BIURET AND RELATED COMPOUNDS

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I. Introduction

Biuret, discovered over one hundred years ago, is the parent compound of a large and interesting class of organic substances. It was first obtained by Wiedemann (428), who, working in Magnus' laboratory in Berlin, isolated the new compound from the products of the thermal decomposition of urea or urea nitrate. On the basis of its ultimate composition, Wiedemann correctly interpreted its formation by the loss of ammonia from two molecules of urea, and therefore proposed the name which is still in use today. However, because of the numerical values of the equivalent weights of carbon and oxygen then in use, the reaction was represented by the following equation:

$$2C_2H_4N_2O_2 \rightarrow C_4H_5N_3O_4 + NH_3$$

Wiedemann also referred to the characteristic red color obtained upon the addition of copper sulfate to alkaline solutions of biuret. This first memoir on the subject, published only twenty years after Wöhler's classical synthesis of urea, thus contains no less than the discovery of biuret, that of the biuret reaction, and the basis of our present-day nomenclature of this section of organic chemistry. The fact that fifteen years elapsed before the next paper appeared on this subject (118) underlines the pioneer character of Wiedemann's work. Trisubstituted biurets had indeed been obtained in 1858 by the hydrolysis of *N*,*N*,*N*-trialkylisocyanuric acids (171, 241), but the true nature of these products was recognized only many years later (262). By 1880, however, more than twenty investigations had dealt with the chemistry of biuret (17, 18, 26, 93, 102, 103,

118, 163, 165, 171, 192, 193, 197, 198, 241, 262, 283, 305, 319, 340, 419, 428, 431) and henceforth work continued apace with ever-increasing momentum. It is of interest, incidentally, that Baeyer recognized biuret in 1864 as one of the degradation products of barbituric acid (17, 18) in the course of his classical researches on uric acid.

A general synthesis of biuret by the ammonolysis of allophanic esters was discovered by Hofmann in 1871 (192, 193) and was extended to the production of substituted homologs in the same year. Thiobiuret was obtained by Wunderlich and Hecht (178, 436) in 1886; their general method, employing cyanoureas as starting material, was readily applicable to the preparation of substituted thio- and dithiobiurets. Another early achievement is Glutz's first preparation, in 1870, of 1-aryldithiobiurets by the "isoperthiocyanic" acid synthesis (163). In marked contrast the parent compound, dithiobiuret, eluded all efforts to prepare it until 1945 (364). Because of the numerous unsuccessful attempts at its synthesis (133, 141, 145, 146, 150, 178, 436), its existence began to be doubted; as late as 1943 some unknown factor was suspected of hindering the replacement of both oxygen atoms in biuret by sulfur, unless other substituents are present to stabilize the resulting molecule (177). Today, dithiobiuret is commercially available.

In recent years the chemistry of biuret and related compounds has attracted increasing attention. Physiological and potential chemotherapeutic properties of numerous derivatives have been studied, and possible technical applications, particularly in the field of plastics and resins, are embodied in an extensive patent literature. The subject has not been previously reviewed.

The present article attempts to present a comprehensive review of the existing knowledge concerning biuret and related compounds. Because of the early discoveries, and the sustained interest of numerous investigators in this field over the years, studies in this subject reflect, to a considerable degree, the development of organic chemistry as a whole. Much fundamental work had been carried out by the turn of the present century; it seemed therefore necessary to include, as far as possible, all significant contributions to biuret chemistry since the discovery of the parent compound in 1847. The later literature is covered, through *Chemical Abstracts*, up to January 1954, but a number of later papers have also been noticed. The review concludes with a brief summary of the biological properties of biurets and a short consideration of the biochemically important biuret reaction.

II. Biuret

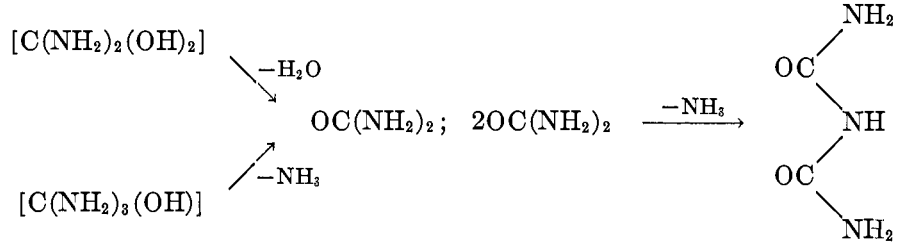
A. Nomenclature and structure

Biuret (I) and its sulfur-containing analogs (II, III) may be related to several simpler compounds. It is formally derived from carbonic acid (IV), the ultimate parent compound of this branch of organic chemistry, by the replacement of the two hydroxyl groups by one amide and one ureido grouping. It may also be regarded as the ureide of the hypothetical carbamic acid (V) or as the amide of allophanic acid (VI). Its relation to formamide (VII) or to ammoniadicarboxylic

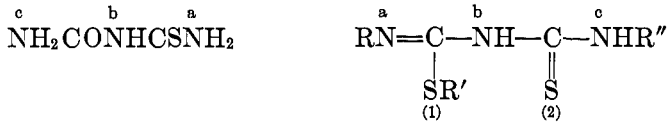
acid (VIII) is occasionally reflected in the nomenclature of the earlier literature. These relationships serve not only for the formal comparison of these compounds but provide in some cases the basis of useful syntheses and degradations of biuret.

$\overset{1}{N}H_2\overset{2}{C}O\overset{3}{N}H\overset{4}{C}O\overset{5}{N}H_2$	$NH_2CSNHCONH_2$	$NH_2CSNHCSNH_2$
I	II	III
Biuret	Thiobiuret	Dithiobiuret
HOCOOH	$[NH_2COOH]$	$[\overset{4}{N}H_2\overset{3}{C}O\overset{2}{N}H\overset{1}{C}OOH]$
IV	V	VI
Carbonic acid	Carbamic acid	Amide of allophanic acid
$HCONH_2$	$NH(COOH)_2$	$\overset{1}{N}H_2\overset{2}{C}O\overset{3}{N}H\overset{4}{C}O\overset{5}{N}H\overset{6}{C}O\overset{7}{N}H_2$
VII	VIII	IX
Formamide	Ammoniadicarboxylic acid	Triuret

In Franklin's nitrogen system of compounds (48, 129, 130) biuret is classified as an aquo-ammonodicarbonic acid, derived from hypothetical orthocarbonic acid as follows:



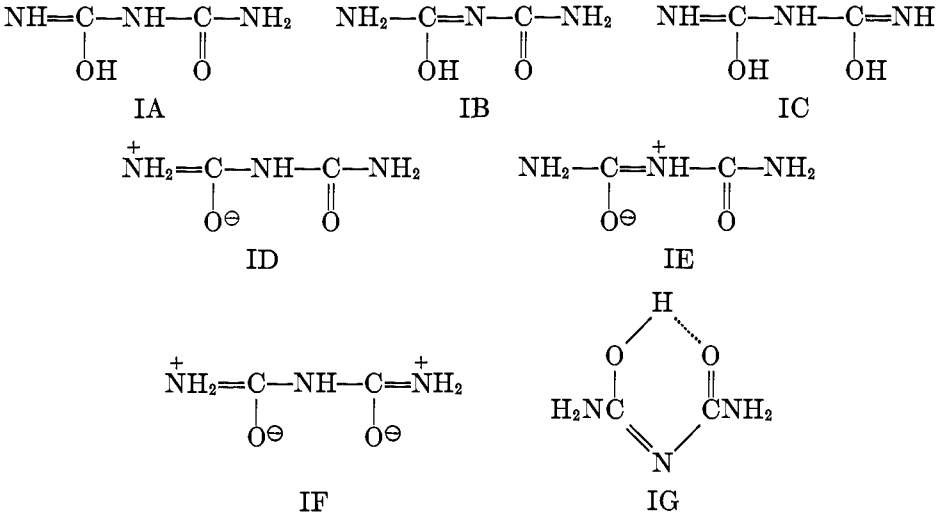
The name biuret, given to the parent compound by its discoverer (428) in 1847, expresses its formation from two molecules of urea. It seems gratifying that the original trivial name serves satisfactorily as the basis of our present-day nomenclature. The system of numbering adopted by *Chemical Abstracts* (*cf.* formula I) provides an unambiguous name for any biuret or thiobiuret derivative. The positions of the atoms in triuret (IX) are similarly defined. Two older modes of numbering thiobiurets and dithiobiurets, due to Dixon (99) (*cf.* formula Ia) and Johnson (209) (*cf.* formula Ib), respectively, which have been adopted, though not consistently, in Beilstein's handbook, lack the precision and conciseness of the present-day notation and are no longer employed.



Ia Ib

Attention may also be drawn to Maquenne's proposals (248), published in 1893, for a comprehensive system of nomenclature covering both open-chain and cyclic ureide structures. Within the framework of this scheme, biuret derivatives were classified into "biuramines," "biuramides," "biurimides," and "biuraminides," the three nitrogen atoms being duly distinguished by superscripts. The suggested rules did not find favor, however, and were never widely adopted in naming ureas, biurets, or related structures.

As in the case of urea, it is difficult to assign a definite and detailed structure to biuret. Some of its structural features undoubtedly vary with the nature of the environment. Biuret may, of course, exist in the form of a partly, or fully enolized tautomeride (IA, IB, or IC). The most satisfactory representation is probably a resonance hybrid, with contributions from the conventional (I) and various "zwitter-ion" forms, such as ID, IE, or IF. A possible modification of the structure due to hydrogen bonding, particularly in the solid state, might indeed give rise to cyclic structures such as IG (177).



Owing to the complex morphology of the biuret crystal, attempts to elucidate the structure by x-ray analysis have so far proved unsuccessful (177).

B. SYNTHESIS

1. Action of heat on urea

When urea or urea nitrate is melted and kept at 150–170°C. for several hours, the resolidified melt consists of unchanged starting material, cyamelide, cyanuric acid, and biuret. After the removal of the former products, the biuret may be isolated by evaporation of the aqueous extracts. Although biuret was discovered by this reaction (428) and was prepared by this method in early researches (118, 193, 340), its use for preparative purposes is limited; the reaction occurs slowly, the temperature is difficult to control, and the formation of large quantities of

by-products results in small yields. The use of phenol as solvent (18) ensures a more even distribution of heat, but improves the method only slightly (333).

Better results are obtained when chlorine gas is passed through melted urea at 150°C. (197). Heating for 1 hr. under these conditions affords, in addition to much cyanuric acid, a 48 per cent yield of biuret. Adequate experimental details were first given by Thiele and Uhlfelder (383).

In the same year Schiff (333) described the superior results obtainable in the decomposition of urea hydrochloride. The formation of small quantities of biuret by prolonged heating of this salt on the water bath had in fact been previously noted (102). Yields of 45 per cent of biuret were obtained by heating urea with simultaneous passage of hydrogen chloride. A more convenient method of removing the cyanuric acid, hitherto precipitated with lead acetate, consisted in heating the crude mixture of products with alcoholic potassium hydroxide, in which potassium cyanurate was insoluble.

Related reactions which yield small quantities of biuret, presumably from intermediately formed urea, include the evaporation of aqueous solutions of cyanic acid or nitrourea (421). The slow formation of biuret in solutions of urea at temperatures as low as 60°C., or at room temperatures under reduced pressure, has also been observed (266).

In spite of the difficulties, the pyrolysis of urea has been developed for the manufacture of biuret and is suitable, under carefully controlled conditions, for the large-scale preparation of this compound. Keeping urea at 130–205°C. and at pressures less than 200 mm. of mercury for several hours, for example, affords up to a 50 per cent yield of biuret (176). The use of catalysts (such as ammonium phosphomolybdate or vanadate, arsenious oxide, selenium molybdate, or sodium perborate) allows the reaction time to be reduced to less than 1 hr. (153). Improved results have been claimed for a process in which the liberated ammonia is displaced by a stream of toluene vapor, introduced below the surface of molten urea (268). Alternatively, fused urea is stirred at 120–130°C. until the mass solidifies and stirring is no longer possible. The product is separated by means of hot water into biuret, triuret, and unchanged urea. In order to avoid overheating, the melting point of the fused mixture may be lowered by the addition of diluents such as diethylformamide, diethanolamine, or triethanolamine (363).

In several reactions the production of small quantities of biuret is no doubt due to the deamination of primarily formed urea. This explanation may account for the presence of biuret amongst the products of the electrolysis of concentrated aqueous ammonia between carbon electrodes (253). Traces of biuret formed in the thermal decomposition of nitrourea (89) arise probably from intermediate urea by the usual mechanism.

The zinc oxide-catalyzed hydration of hydrogen cyanide at 200°C. yields biuret amongst other compounds; the reaction is believed to proceed by the following stages (326, 327):

$$HCN + H_2O \rightarrow NH_2CHO$$

$$2NH_2CHO + H_2O \rightarrow NH_2CONH_2 + CO_2 + 2H_2$$

$$2NH_2CONH_2 \rightarrow NH_2CONHCONH_2 + NH_3$$

The thermal decomposition of formamide is known to take place in two ways:

$$CO + NH_3 \leftarrow HCONH_2 \rightarrow HCN + H_2O$$

Formamide

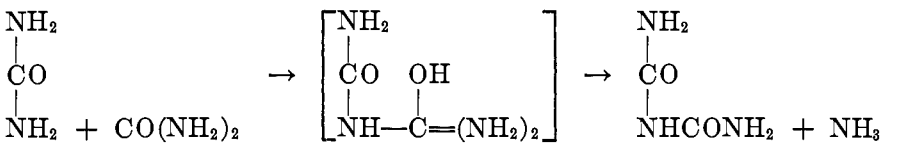
In the presence of catalysts such as zinc, however, a small proportion of the formamide decomposes to urea, and thence to biuret (344):

$$HCONH_2 \rightarrow HNCO + H_2$$

$$HNCO + NH_3 \rightarrow NH_2CONH_2; \rightarrow \text{biuret}$$

Biuret and triuret, obtained in addition to 5-hydantoinacetic acid when urea and fumaric acid are kept at 135–140°C. for 2.5 hr. (207), are in fact probably direct decomposition products of urea.

The mechanism of the thermal conversion of urea into biuret and cyanic acid has caused a good deal of speculation. Chattaway had tentatively postulated a preliminary condensation of the amino group of one molecule of urea with the carbonyl group of a second, the hypothetical intermediate giving rise to biuret by loss of ammonia; a repetition of this process, followed by cyclization, accounts for the formation of the cyanuric acid (68).



Biuret

Werner, in the first detailed study of the thermal decomposition of urea (422, 423), adduced evidence for a preliminary dissociation of urea into ammonia and cyanic acid; the further (and incidentally reversible) reaction of the latter with urea was considered responsible for the synthesis of biuret. Simultaneous trimerization of cyanic acid produced cyanuric acid as the second main product of this reaction.

$$NH_2CONH_2 \rightarrow NH_3 + HCNO$$

$$NH_2CONH_2 + HCNO \rightleftharpoons NH_2CONHCONH_2$$

$$3HCNO \rightarrow (HCNO)_3$$

Cyanuric acid

The formation of traces of biuret in the pyrolysis of oxamide was similarly accounted for (425). Werner's observations (424), and previous syntheses of biuret from urea and cyanic acid (or reagents serving as a source of this compound) (102, 118), appear to lend support to the above interpretation.

2. Action of inorganic halides on urea

Urea is deaminated smoothly to biuret by a number of inorganic acid halides. (*a*) The action of thionyl chloride, first erroneously reported to afford cyana-

mide (260), was shown by Warren and Wilson to yield biuret as the sole product (416; see also 426).

In a detailed study of this reaction Haworth and Mann observed the formation of both biuret and triuret, the proportion of the latter increasing with the amount of thionyl chloride employed. The resulting mixture was readily separated into its pure constituents by crystallization from water. Optimum conditions were found for the preparation of both biuret and triuret, and were considered by the authors to provide the best known method for the preparation of these compounds (177; see also 420).

(*b*) As expected, sulfuryl chloride reacts analogously to thionyl chloride, though more vigorously. Under carefully controlled conditions biuret is isolated (from an intermediate complex, $C_4H_{10}O_4N_8S \cdot 3H_2O$, of unknown constitution), but cyanuric acid becomes the main product at higher temperatures. It is of interest that biuret, once formed, does not undergo further deamination on treatment with boiling thionyl or sulfuryl chloride (177).

(*c*) Similarly, 1 mole of chlorosulfonic acid converts 2 moles of urea to biuret in the cold. The use of an excess of the reagent yields only cyanuric or aminosulfonic acid, together with a trace of triuret, depending on the conditions employed (177).

(*d*) Finally, phosphorus trichloride at 100°C. is a suitable deaminating agent and affords good yields of biuret, in addition to an amorphous substance (possibly triuret) (419).

Of the numerous syntheses of biuret, the following three methods (Sections 3–5) clearly confirm the correctness of the structure assigned to this compound.

3. Interaction of urea and cyanic acid

Small quantities of biuret are formed when cyanic acid gas is passed into melted urea (118). Better results are obtained when a dilute solution of urea and potassium cyanate, acidified with acetic acid, is slowly evaporated (102; see also 424).

$$\underset{\text{Urea}}{NH_2CONH_2} + HCNO \rightarrow \underset{\text{Biuret}}{NH_2CONHCONH_2}$$

In the enzymatic hydrolysis of urea by urease, traces of biuret are believed to be formed by this mechanism (115).

4. Ammonolysis of allophanic esters and related compounds

Treatment of melted ethyl allophanate with gaseous ammonia affords small yields of biuret (197); the reaction occurs readily and completely when the ester is heated with aqueous ammonia at 100°C. in closed vessels (193). Anhydrous liquid ammonia, or liquid ammonia containing 2 per cent of water, on the other hand, is without effect, the allophanate being recovered unchanged (84).

$$\underset{\text{Ethyl allophanate}}{NH_2CONHCOOC_2H_5} + NH_3 \rightarrow \underset{\text{Biuret}}{NH_2CONHCONH_2} + C_2H_5OH$$

The analogous conversion of the dithiobenzyl ester of imidodicarboxylic acid into biuret and benzylthiol (133) may also be included under this heading.

$$NH(COSR)_2 + 2NH_3 \rightarrow NH_2CONHCONH_2 + 2RSH$$

The interaction of allophanic azide (prepared from aminobiuret and nitrous acid) (383) with dilute, concentrated, or liquid ammonia affords good yields of biuret (242). The reported formation (383) of tetruret by this reaction, however, has not been confirmed (242).

Finally, the conversion of allophanyl chloride to the amide provides yet another unambiguous route to biuret; the reaction is readily effected by aqueous ammonia and yields, in addition to the main product, small quantities of urea and triuret (57).

$$\underset{\text{Allophanyl chloride}}{NH_2CONHCOCl} + 2NH_3 \rightarrow NH_4Cl + \underset{\text{Biuret}}{NH_2CONHCONH_2}$$

$$3NH_2CONHCOCl + 5NH_3 \rightarrow 3NH_4Cl + \underset{\text{Triuret}}{2(NH_2CONH)_2CO}$$

$$NH_2CONHCOCl + H_2O \rightarrow HCl + CO_2 + NH_2CONH_2$$

5. Hydrolysis of cyano compounds

The smooth hydrolysis of suitable cyano compounds to biuret provides confirmation for the correctness of the structure of the latter.

Thus, biuret is obtainable from cyanourea by the action of dilute sulfuric or nitric acid; hydrochloric acid is less suitable (26).

$$NH_2CONHCN + HOH \xrightarrow{H_2SO_4} NH_2CONHCONH_2$$

Dicyanamide, in the form of the hydrochloride (250, 352) or the sodium salt, is readily hydrolyzed to biuret by hot water or concentrated hydrochloric acid, respectively (247).

$$NH(CN)_2 + 2HOH \rightarrow NH_2CONHCONH_2$$

6. Ammonolysis of nitrobiuret

Treatment of nitrobiuret with concentrated ammonia in closed vessels at 100°C. affords 60 per cent yields of biuret, possibly by a preliminary rearrangement of nitrobiuret into nitramide and the hypothetical dicyanic acid, followed by combination of the latter with ammonia (90; but compare 421).

$$\underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} \rightleftharpoons \underset{\text{Dicyanic acid}}{NH_2CONCO} + NH_2NO_2\ (N_2O + H_2O)$$

$$NH_2CONCO + NH_3 \rightarrow NH_2CONHCONH_2$$

The reaction, which occurs equally readily with primary and secondary amines, is a convenient route to substituted biurets (90) (*cf.* Sections V,A,1(i) and V,C,1(c)).

7. Dealkylation of O-alkylisobiurets (cf. Section VI,A)

8. Hydrolysis of biuret-ω-carboxylic acid chloride

The following synthesis of biuret by hydrolyzing biuret-ω-carboxylic acid chloride (prepared from carbamyl chloride at 60°C.) has been claimed (409).

$$3NH_2COCl \rightarrow NH_2CONHCONHCOCl + 2HCl$$

$$NH_2CONHCONHCOCl + H_2O \rightarrow NH_2CONHCONH_2 + CO_2 + HCl$$

Biuret-ω-carboxylic acid chloride — Biuret

9. Ammonolysis of hydroxyoxamide

A quantitative yield of biuret is obtained when acetylhydroxyoxamide (337) is boiled with dilute aqueous ammonia. The reaction probably proceeds as follows (*cf.* Section V,A,1(f)) (290):

$$NH_2COCONHOCOCH_3 \rightarrow CH_3COOH + [NH_2CONCO]$$

Acetylhydroxyoxamide

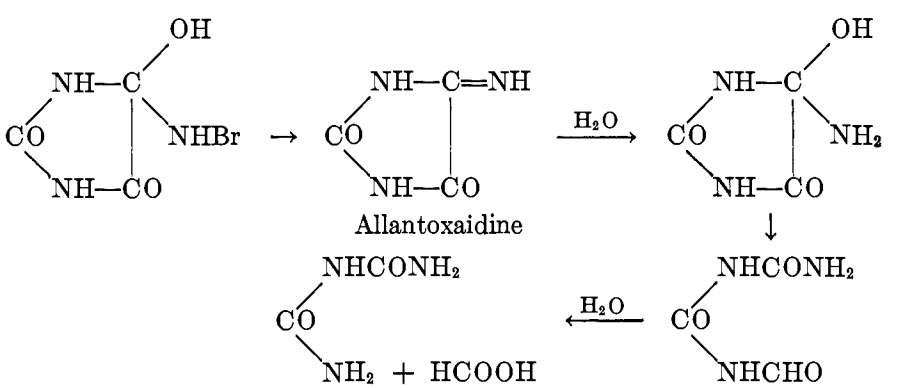
$$[NH_2CONCO] + NH_3 \xrightarrow{\text{heat}} NH_2CONHCONH_2$$

10. From heterocyclic compounds

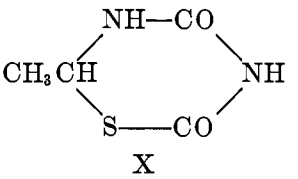
Biuret and its derivatives have often been obtained amongst the degradation products of heterocyclic compounds, particularly purines. As early as 1864 Baeyer (17) reported that tribromoacetylurea, produced in the oxidation of purines by bromine, is decomposed by aqueous ammonia into bromoform and biuret.

$$CBr_3CONHCONH_2 + NH_3 \rightarrow CHBr_3 + NH_2CONHCONH_2$$

Biuret is the main product when uric acid is oxidized with 5 per cent alkaline permanganate (29). Allantoxaidine (5-iminohydantoin) is split into biuret and formic acid by boiling water or acids (43, 305) and into biuret and cyanuric acid by hypobromous acid (43). Biltz and Robl (43) interpreted this reaction, and the analogous degradation of 5-hydroxy-5-bromoaminohydantoin, as follows:



The fission of the thiadiazine X (obtained in the condensation of ethylene thiocyanohydrin and aminoethanol) by ammonia produces biuret in fair yields (410).



X

C. PHYSICAL PROPERTIES

The density of solid biuret at −5°C. is 1.467 (27). When crystallized from aqueous solution (for its solubility *cf.* 193 and 322), biuret forms a hydrate which was originally reported to contain 1 mole of water of crystallization (193, 428). According to the results of recent measurements, however, the solvated crystals have the composition $5C_2H_5O_2N_3 \cdot 4H_2O$ (322). The water of crystallization is partially lost on storage at room temperature (29), and is completely removed at 110°C. (193). The anhydrous compound, also obtained by crystallization from alcohol (428), is hygroscopic and rapidly absorbs water from the atmosphere (29). An addition compound with hydrogen peroxide, $5C_2H_5O_2N_3 \cdot H_2O_2$, is also on record (375). The results of micro wetting point–melting point procedures suggest the existence of molecular compounds of biuret with methyl *p*-aminobenzoate, but not with sulfanilamide (351).

The optical properties of biuret crystals have been studied in detail (254) and are summarized in table 1. The presence of traces of biuret influences the crystalline form of ammonium chloride; the salt crystallizes in the form of cubes under these conditions (156).

Measurements of the dielectric constant of aqueous solutions of biuret (95)

TABLE 1

Optical properties of biuret

Crystal habit	Elongated plates from ethanol
Refractive indexes (at 5461 Å.):	
α	1.403 ± 0.005
β	1.616
γ	1.624
Birefringence	0.221
Optical axial angle (5461 Å.):	
$2H_a$	17°
$2V$	18°
Dispersion	$v > r$*
Elongation:	
Bx_a	$\parallel a$
Bx_o	Λ 28 ± 3°α
Probable crystal system	$2H_a$ = 19.5° (4358 Å.) = 13° (6908 Å.)

* Greater for blue than for red light.

and of the dielectric increment[1] of biuret are on record (73). Data are also available concerning its electrocapillary function (164), its piezoelectric behavior (186), and the magnetic susceptibility of its carbonyl group (282).

The conductivity of biuret and its effect on that of solutions of boric acid have been measured (53). When added to an inert electrolyte, biuret raises the potential (by 0.2 v.) at which oxygen is evolved at the platinum anode, but the origin of this phenomenon is obscure (187).

A study of the phase diagram for the system water–biuret reveals a eutectic (ice–biuret hydrate) at 0°C. in the region of 0.084–0.48 per cent of biuret. Under pressure, a transition point is observed at 112.5°C., at which two solid phases, biuret and biuret hydrate, are in equilibrium with the solution (322). The solubility of biuret in water, small at 0°C., increases rapidly with rise in temperature (322).

Temperature, °C.	25	50	75	105.05 (b.p.)
Solubility, grams of biuret in 100 g. of solution	2.01	7	20	53.5

Melting-point determinations of urea–biuret mixtures of varying compositions indicate a transition point at 114.5°C. (36 per cent urea) and eutectics at 106°C. and 111°C., the former being metastable. The results reveal the existence of a molecular compound consisting of two molecules of urea with one of biuret (321). Phase-rule studies have also been carried out on the following systems: biuret–ammonia; biuret–ammonium bicarbonate; biuret–urea–ammonium bicarbonate; biuret–urea–dicyandiamide (206).

D. CHEMICAL PROPERTIES

1. General

Biuret shows amphoteric character, but both its basic and acidic properties are feeble. Thus, its addition compounds with acids, such as hydrochloric acid ($2C_2H_5O_2N_3 \cdot HCl$) (118) and cyanuric acid (183), are not stable in aqueous solution; the hydrochloride, for example, is almost completely hydrolyzed in decimolar solution (118, 434).

The acidic properties of biuret are reflected in the existence of monopotassium and tripotassium salts; these are formed, in liquid ammonia, by the addition of the appropriate proportion of potassium amide to biuret but are immediately decomposed by water (49).

$$C_2H_5O_2N_3 + KNH_2 \rightarrow C_2H_4O_2N_3K + NH_3$$

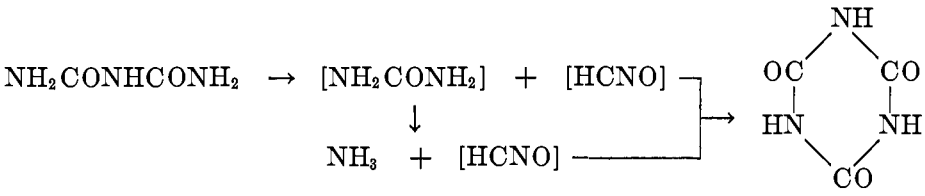
$$C_2H_5O_2N_3 + 3KNH_2 \rightarrow C_2H_2O_2N_3K_3 + 3NH_3$$

Addition compounds with alkalies (e.g., $C_2H_3O_2N_3$ + NaOH or KOH), which are rapidly hydrolyzed by water, can also be prepared (333) (*cf.* table 9). Complex salts are discussed in connection with the biuret reaction (*cf.* Section XVI).

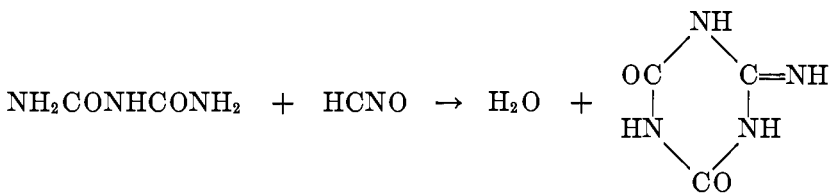
[1] The dielectric increment, δ, is defined as the change in the dielectric constant of the solution per mole of solute per 1000 g. of water.

2. Action of dry heat

Fused biuret decomposes with evolution of ammonia; its melting point therefore depends somewhat on the rate of heating (17, 193, 332). The solid residue consists principally of cyanuric acid (17, 193). The thermal decomposition near 200°C. was examined in some detail by Werner (422, 423). The reaction was shown to involve the preliminary dissociation of biuret into cyanic acid and urea. Under suitable conditions the presence of urea in the fusion residue may be demonstrated; on continued heating most of it decomposes further into ammonia and cyanic acid, which polymerizes in turn to cyanuric acid. At the same time, condensation of biuret with cyanic acid, with elimination of water, gives rise to small quantities of ammelide (which had previously been identified incorrectly as cyanuric acid triureide) (174).



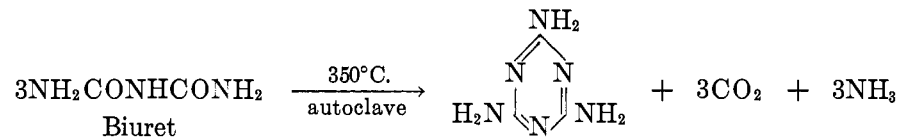
Cyanuric acid



Ammelide

An alternative interpretation, involving the intermediate formation of dicyanic acid (NH_2CONCO), has been advanced by Das Gupta (87).

At higher temperatures biuret (like urea, amidinourea, cyanuric acid, ammeline, and ammelide) is converted into melamine. Pyrolysis at 350°C. in an autoclave, preferably in the presence of ammonia, for example, affords 55 per cent yields of the triazine (8).



Melamine

3. Hydrolysis by acids and alkalies

Anhydrous biuret slowly absorbs hydrogen chloride at 100°C. and yields the hydrochloride, $2C_2H_5O_2N_3 \cdot HCl$ (118); at higher temperatures the acid promotes

complete decomposition, with elimination of ammonia and carbon dioxide, to cyanuric acid, urea, and guanidine (118).

$$NH_2CONHCONH_2 \rightarrow NH_2CONH_2 + HCNO$$

$$NH_2CONHCONH_2 \rightarrow \underset{\text{Guanidine}}{NH_2C(=NH)NH_2} + CO_2$$

$$\underset{\text{Biuret}}{2NH_2CONHCONH_2} \rightarrow 2NH_3 + \underset{\text{Cyanuric acid}}{(CHON)_3}$$

The hydrolysis of biuret by sulfuric acid is catalyzed by mercuric sulfate (397).

Warm concentrated nitric acid decomposes biuret slowly into cyanuric acid and finally urea nitrate (118). At low temperatures, however, biuret is successively nitrated to mononitrobiuret and dinitrobiuret (383) (*cf.* Sections III,A and III,B).

Complete hydrolysis of biuret is also effected in alkaline solution; baryta, for example, yields urea, carbon dioxide, and ammonia (118).

$$NH_2CONHCONH_2 + H_2O \rightarrow NH_2CONH_2 + CO_2 + NH_3$$

4. Oxidation and reduction

In conformity with the presence of two amide groups in the molecule, biuret evolves 2 gram-atoms of nitrogen when treated with alkaline sodium hypobromite (75, 182, 183). There is evidence for a preliminary decomposition and rearrangement into semicarbazide, which decomposes subsequently into carbon dioxide, ammonia, hydrazine, and finally nitrogen; hydrazine can in fact be isolated as the dibenzal derivative (85, 86).

Nitrous acid, in acetic acid solution, decomposes biuret similarly, with evolution of one equivalent of nitrogen. In the presence of hydrochloric acid, however, two or all three nitrogen atoms are liberated in the gaseous form, depending on the concentration of the acid (301).

The attempted electrolytic reduction of biuret in acid solution (under conditions that had proved suitable for numerous amides) was not successful (224).

5. Attempted alkylation

Attempts to introduce alkyl groups into biuret directly have so far not been successful. When treated with methyl iodide in methanol at 120–130°C. under pressure for several hours, biuret is decomposed into ammonia and small quantities of methylamine (182). The methylation with diazomethane appears to occur only incompletely (184).

6. Halogenation

Treatment of aqueous solutions of biuret, at 60–70°C., with chlorine precipitates 1,5-dichlorobiuret (*cf.* Section IV,B). Under similar conditions acetylbiuret

yields a dichloro derivative, which is regarded as 1-acetyl-5,5-dichlorobiuret (88).

7. Reaction with hydroxy compounds

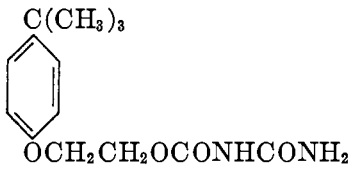
When heated with ethanol in the presence of hydrogen chloride at 140–145°C. under pressure, biuret is converted into ethyl allophanate and ammonia (291).

$$\underset{\text{Biuret}}{NH_2CONHCONH_2} + C_2H_5OH \rightarrow \underset{\text{Ethyl allophanate}}{NH_2CONHCOOC_2H_5} + NH_3$$

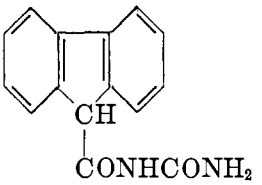
This reaction is, of course, the reverse of the synthesis of biuret from allophanates (*cf.* Section II,B,4) (193, 197).

Numerous allophanates and related compounds have been obtained by a modification of this method, in which biuret or its thio or imido analogs are heated with the appropriate hydroxy compound under reduced pressure. Condensation of biuret and 1-(ω-hydroxyethoxy)-4-(*tert*-butyl)benzene at 140–150°C. and 100–200 mm. pressure, for example, affords 95 per cent yields of the allophanate (XI). As expected, dihydroxy compounds give mixtures of mono- and diallophanates (4, 5). Some of these products are useful wear- and corrosion-reducing agents when added, in 0.1–2 per cent concentration, to high-pressure lubricating oils.

The allophanyl group may be similarly introduced into other structures containing a reactive hydrogen atom: condensation of biuret with suitable compounds occurs with elimination of ammonia and affords varying yields of the allophanyl derivative. Successful results have been claimed for certain hydrocarbons, such as fluorene and indene, carboxylic acids and their esters, malonic and acetoacetic esters, and a number of heterocyclic compounds. A few typical reaction products are represented below (3).

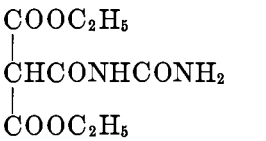


XI

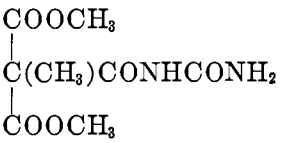


9-Allophanylfluorene

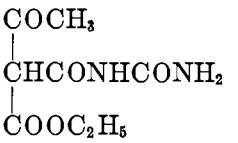
2-Allophanylthiophene



Diethyl α-allophanylmalonate



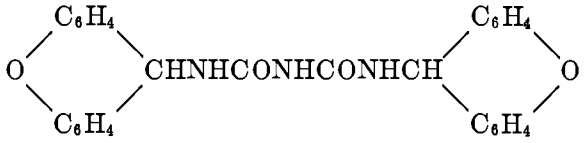
Dimethyl α-methyl-α-allophanylmalonate



Ethyl α-allophanylacetoacetate

Xanthydrol, the well-known reagent for the identification of amides and ureas,

condenses with the two amide groups of biuret as expected, to yield *sym*-dixanthylbiuret (124, 125).



sym-Dixanthylbiuret

8. Reaction with aldehydes and ketones

Dimethylolbiuret may be prepared by the interaction of biuret and an excess of formaldehyde at 100°C. in aqueous solution at pH 7.6. In acidic media, however, viscous solutions of a polymeric condensation product are obtained instead.

$$NH_2CONHCONH_2 + 2HCHO \rightarrow HOCH_2NHCONHCONHCH_2OH$$

Dimethylolbiuret

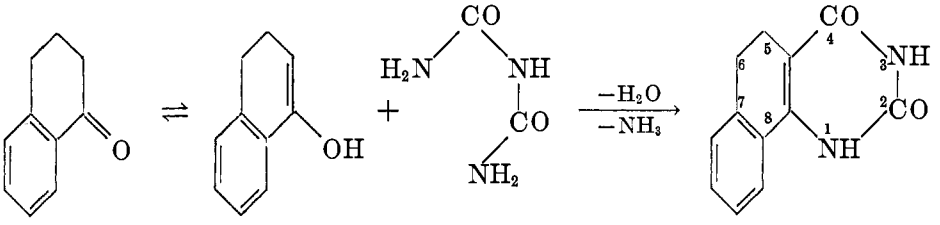
$$\downarrow CH_3OH,\ HCl$$

$$CH_3OCH_2NHCONHCONHCH_2OCH_3$$

1,5-Dimethoxymethylbiuret

Dimethylolbiuret, or 1,5-dimethoxymethylbiuret prepared therefrom, is readily convertible, on heating with small quantities of maleic or phthalic acid, into resinous products which are useful as coating compositions (354; compare also 334).

The condensation of α-tetralone (1-oxo-1,2,3,4-tetrahydronaphthalene) with urea in the molecular ratio 1:2 at 180°C. appears to involve the preliminary conversion of urea to biuret, which reacts with the ketone to form 1,2,3,4,5,6-hexahydro-2,4-dioxo-7,8-benzoquinazoline in 60 per cent yields. Support for this view is provided by the observation that biuret may take the place of urea in this reaction (107).



α-Tetralone

9. Reaction with amines

The interaction of biuret and aniline at 160–180°C. produces diphenylurea and small yields (9–11 per cent) of phenylbiuret. The preliminary rearrangement of

biuret into dicyanic acid on the one hand, and into urea on the other (*cf.* 90), has been suggested as involved in the mechanism of this change (87).

$$\underset{\text{Biuret}}{NH_2CONHCONH_2} \longrightarrow \begin{cases} NH_3 + NH_2CONCO \\ NH_2CONH_2 + HCNO \end{cases}$$

$$\underset{\text{Dicyanic acid}}{NH_2CONCO} + C_6H_5NH_2 \rightarrow \underset{\text{Phenylbiuret}}{C_6H_5NHCONHCONH_2}$$

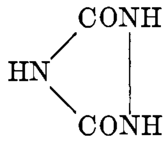
$$HCNO + C_6H_5NH_2 \rightarrow \underset{\text{Phenylurea}}{C_6H_5NHCONH_2} \rightarrow \underset{\text{Diphenylurea}}{(C_6H_5NH)_2CO}$$

An early report by Hofmann records the formation of a diphenylbiuret, of undetermined structure, in this reaction (193).

10. Condensation with hydrazines

Biuret condenses with hydrazine salts at 150–160°C. (284) or with an excess of hydrazine hydrate at 110°C. (374) to form good yields of urazole together with small quantities of cyanuric acid and biurea ($NH_2CONHNHCONH_2$).

$$\underset{\text{Biuret}}{NH_2CONHCONH_2} + NH_2NH_2 \rightarrow NH_3 + \underset{\text{Aminobiuret}}{[NH_2CONHCONHNH_2]}$$

$\rightarrow 2NH_3 +$ 

Urazole

The correctness of the suggested mechanism has been confirmed by performing the reaction at lower temperatures, when the postulated intermediate aminobiuret was isolated as the benzylidene derivative (374).

Phenylhydrazine affords the corresponding *N*-phenyl-substituted urazole (356), but ring-closure cannot occur in the reaction involving two equivalents of *as*-diphenylhydrazine at 190°C.; here tetraphenyldiaminobiuret is obtained almost quantitatively (252).

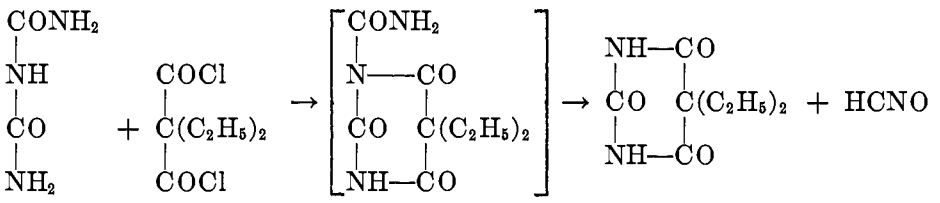
$$NH_2CONHCONH_2 + 2(C_6H_5)_2NNH_2 \rightarrow$$

$$2NH_3 + \underset{\text{1,5-Bis(diphenylamino)biuret}}{(C_6H_5)_2NNHCONHCONHN(C_6H_5)_2}$$

11. Condensation with acid chlorides

When treated with an excess of phosgene in closed vessels at 60°C. two molecules of biuret are claimed to condense to form carbonyldibiuret (332, 340) (*cf.* Section XIV).

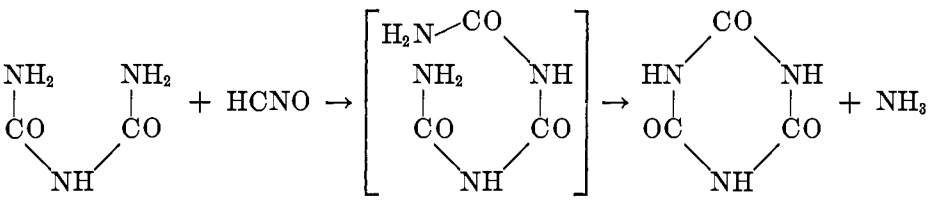
Condensation of biuret with diethylmalonyl dichloride at 120–130°C. yields 5,5-diethylbarbituric acid as follows (251):



5,5-Diethyl-barbituric acid

12. Condensation with carbonic acid derivatives

Just as urea adds the elements of cyanic acid to form biuret, biuret condenses with another molecule of cyanic acid, but cyclizes immediately, with loss of ammonia, to yield cyanuric acid. Potassium cyanate or urethan may serve as the source of the cyanic acid (23).



Cyanuric acid

The reaction between biuret and nitrobiuret has been claimed to afford small yields of tetruret (90), but Werner and Gray have failed to confirm this result (420).

E. BIURET IN ORGANIC TECHNOLOGY

A large number of useful condensation products and emulsions, incorporating biuret in some form or other, have been described in the patent literature. The published information is briefly summarized in table 2. In certain cases biuret may partially replace urea in the manufacture of urea–formaldehyde condensation products (161).

A finely ground mixture of urea and biuret has been proposed as a blowing agent for sponge rubber. The effectiveness of this mixture is due to evolution of ammonia during the vulcanization. Evolution of this gas is claimed to occur at a lower temperature from this mixture than would take place from either constituent (345).

Copper phthalocyanine dyes are obtainable in excellent yields by heating a phthaloyl halide with either urea or biuret in the presence of a copper salt (376).

TABLE 2

Condensation products of biuret

Biuret condensed with	Product	Uses	References
Boric acid at 100–160°C.	Water-soluble resins	Adhesives or coatings for paper or textile materials	(246)
Formaldehyde, in the presence of a protein (e.g., casein)	Stable aqueous resin emulsions Dry powder, by cautious removal of solvent, redispersed by stirring with water	Adhesives; binding or impregnating agents	(71, 331) (330)
Formaldehyde, lactic acid, and ethylene glycol	Viscous solution, giving flexible films on drying	Coatings for paper, leather, and textiles	(288)
Formaldehyde, phosphoric acid	Condensed on cellulose fabric by curing	Flame-proofing agent for textiles	(123)
Formaldehyde, followed by sulfonated 4,4-dihydroxydiphenylsulfone	Water-soluble condensation product	Tanning agent for leather	(161)
Substituted glyoxalidines	Viscous liquids	Wetting agents, detergents, and emulsifiers; used for breaking petroleum emulsions	(166, 167)
Polyalkylenepolyamides, followed by higher fatty acids (e.g., stearic, oleic) at 160–200°C.	Water-soluble resinous *or* wax-like emulsifiable products	Softening agent for rayon	(200, 293)
Condensation products of aliphatic polyamines and organic acids, esters, or acid chlorides		Mothproofing agents for textiles	(257, 258, 259)
Butylbiuret and formaldehyde	Intermediates for resin formation		(74)

III. Nitro-, Nitroso-, and Aminobiurets

A. NITROBIURET

1. Formation

In 1898, Thiele and Uhlfelder reported the first preparation of nitrobiuret and described the properties of this compound in some detail.

According to its discoverers (383), nitrobiuret is readily obtained in 70–90 per cent yields by slowly stirring biuret into a cooled mixture of concentrated nitric

$$NH_2CONHCONH_2 + HNO_3 \rightarrow \underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} + H_2O$$

and sulfuric acids, and adding the resulting solution to ice. The product may be purified by reprecipitation from its alkaline solution, or by cautious crystalliza-

tion from water below 70°C.; above this temperature the dissolved compound suffers decomposition (see also 90).

An example of the formation of nitrobiuret as a degradation product of a heterocyclic compound is on record, but here again, the mechanism of its production is no doubt the nitration of intermediate biuret; allantoxaidine (5-iminohydantoin), which breaks down into formic acid and biuret on hydrolysis (*cf.* Section II,B,10) (43, 305), reacts analogously with warm concentrated nitric acid, affording good yields of nitrobiuret (42).

The nitration of 1-methylbiuret and 1,1-dimethylbiuret yields 5-nitro-substituted biurets; Davis and Constan (92) based the assigned structures on the observed regeneration of the original alkylbiurets from their nitro derivatives and ammonia. This result was accounted for by the following reaction scheme, involving the intermediate formation of methyldicyanic acid:

$$\underset{\text{1-Methyl-5-nitrobiuret}}{CH_3NHCONHCONHNO_2} \rightarrow CH_3NHCONCO + NH_2NO_2\ (N_2O + H_2O)$$

$$\underset{\text{Methyldicyanic acid}}{CH_3NHCONCO} + NH_3 \rightarrow \underset{\text{1-Methylbiuret}}{CH_3NHCONHCONH_2}$$

If correct, the above mechanism excludes the presence of the nitro group in the 1- or 3-position of the biuret structure, since biuret *or* urea and methylurea, respectively, would be expected to be formed from 1- or 3-nitrobiuret. The same rearrangement accounts for the useful synthesis of 1,1,5-trisubstituted biurets from 1,1-dimethyl-5-nitrobiuret and amines (*cf.* Section V,F) (92).

2. Physical properties

Nitrobiuret consists of a white crystalline powder, decomposing at temperatures variously given between 165°C. and 223°C. It is sparingly soluble in cold water, but more readily soluble in warm water and alcohol (90, 383).

3. Chemical properties

Nitrobiuret acts as a strong acid in aqueous and alcoholic solution. Addition of alcoholic potassium hydroxide to a methanolic solution of nitrobiuret precipitates the potassium salt ($C_2H_3N_4O_4K$). A silver salt ($C_2H_3N_4O_4Ag$) has also been prepared (383).

Above 70°C. water decomposes nitrobiuret into urea, cyanuric acid, nitrous oxide, and carbon dioxide. This reaction, characteristic of primary nitramines,

$$\underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} \rightarrow NH_2CONH_2 + CO_2 + N_2O$$

confirms the position of the nitro group in nitrobiuret in an amino and *not* the imino group of the molecule.

Nitrobiuret is unaffected by alkalies in the cold but is decomposed with evolution of nitrous oxide on warming (90, 383). Boiling alcoholic potash (containing

20 per cent of water) affords potassium allophanate in 32 per cent yield; in the

$$\underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} + KOH \xrightarrow{\text{heat}} NH_2CONHCOOK + H_2O + N_2O$$

Potassium allophanate

presence of more water (50 per cent), however, urea is produced instead (90).

Nitrobiuret is stable towards anhydrous alcohols at the boiling point. In the presence of water, small amounts of the appropriate alkyl allophanates and alkyl carbamates are successively formed (90).

When heated with ammonia at 100°C. in closed vessels, nitrobiuret yields biuret (60 per cent), along with some cyanuric acid and urea. The use of amines in place of ammonia provides a convenient synthesis of substituted biurets (*cf.* Sections V,A,1(i) and V,C,1(c)) (90).

$$NH_2CONHCONHNO_2 + NH_3 \xrightarrow{100°C.} NH_2CONHCONH_2 + H_2O + N_2O$$

Davis and Blanchard (90) explained the chemical properties of nitrobiuret in solution by postulating its simultaneous rearrangement into dicyanic acid and nitramide, and into urea and nitrocyanic acid.

$$\underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} \begin{cases} \nearrow \underset{\text{Dicyanic acid}}{NH_2CONCO} + \underset{\text{Nitramide}}{NH_2NO_2} \; (\rightarrow N_2O + H_2O) \\ \searrow \underset{\text{Urea}}{NH_2CONH_2} + \underset{\text{Nitrocyanic acid}}{OCNNO_2} \; (\rightarrow N_2O + CO_2) \end{cases}$$

The intermediate formation of hypothetical dicyanic acid, which cannot, admittedly, be isolated (421), would account satisfactorily for the reaction of nitrobiuret with alcohols, ammonia, and amines.

Dissolved in concentrated sulfuric acid, nitrobiuret is capable of giving up its nitro group quantitatively and is a suitable nitrating agent for substances that are not decomposed by concentrated sulfuric acid. Thus, aniline is converted to a mixture of the three nitroanilines, and acet-*p*-toluidide yields 4-acetamino-3-nitrotoluene (90).

Reduction of the nitro group of nitrobiuret affords aminobiuret (*cf.* Section III,D) (383), the benzylidene derivative of which may be used for identification purposes (90). Further nitration of nitrobiuret by means of fuming nitric acid yields dinitrobiuret (383).

The supposed formation of triuret or tetruret by the condensation of nitrobiuret with urea or biuret, respectively, has not been confirmed (90, 420).

Nitrobiuret gives a positive nitramide reaction with ferric sulfate and sulfuric acid (383). Freshly prepared aqueous solutions do not give the biuret reaction. After being boiled, the liquid gives a strong biuret test, but yields only urea and cyanuric acid on evaporation (90).

B. 1,5-DINITROBIURET

Nitration of mononitrobiuret with fuming nitric acid at 0°C., followed by spontaneous evaporation of the resulting solution (over concentrated sulfuric acid and soda lime), yields 1,5-dinitrobiuret. Recrystallization from methanol affords the pure product in 80 per cent yield (383).

$$\underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} + HNO_3 \rightarrow \underset{\text{1,5-Dinitrobiuret}}{NH(CONHNO_2)_2} + H_2O$$

This compound, which decomposes explosively at 124°C., is readily soluble in water and common organic solvents. It acts as a strongly dibasic acid, the dipotassium salt ($C_2HN_5O_6K_2$) of which has been described. Like mononitrobiuret, it is completely decomposed by boiling water into carbon dioxide, ammonia, and nitrous acid (383).

C. NITROSOBIURETS

Although nitrosobiuret itself has not been described, a number of substituted nitroso derivatives are known. From an examination of the behavior of alkylbiurets, Biltz and Jeltsch (40) concluded that nitrosation occurred at a 1- or 5-imino position, the 3-imino and 1- and 5-amino groups of the biuret structure being unaffected.

As a rule, nitroso derivatives separate as yellow crystalline solids when dilute aqueous solutions of the appropriate biuret are treated with an excess of nitrogen trioxide or with acidified sodium nitrite at 0°C. They decompose readily and cannot, generally, be purified by crystallization. A number of nitroso derivatives are listed in table 3.

Nitrosobiurets are thermolabile, both in the solid form and in solution. Above 60°C. aqueous solutions of 1-methyl-1-nitrosobiuret, for example, are decolorized with effervescence, decomposition setting in according to the following equation (40):

$$\underset{\text{1-Methyl-1-nitrosobiuret}}{NH_2CONHCON(NO)CH_3} + H_2O \xrightarrow{>60°C.} NH_2CONH_2 + CO_2 + N_2 + CH_3OH$$

TABLE 3

Nitrosobiurets

Biuret Derivative	Melting Point (Decomposition)	References
	°C.	
1-Methyl-1-nitroso-	139–140	(38, 40, 121)
1-Ethyl-1-nitroso-	119–120	(40)
1,3-Dimethyl-1-nitroso-	101	(357)
1,5-Dimethyl-1-nitroso-	108	(40)
1,5-Dimethyl-1,5-dinitroso-	94	(40)
1-Methyl-1-nitroso-5-phenyl-	126	(35)
1,5-Dinitroso-1,3,5-trimethyl-	102	(40)

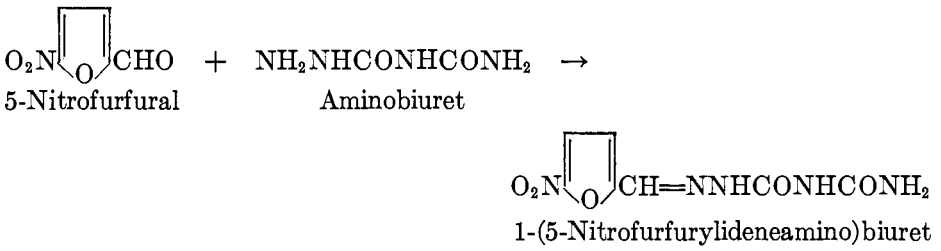
D. AMINOBIURET (ALLOPHANIC ACID HYDRAZIDE)

The reduction of nitrobiuret by zinc and hydrochloric or acetic acid yields aminobiuret. The product is most advantageously isolated (in 40–50 per cent yields) as the benzal derivative, from which aminobiuret may be liberated as the hydrochloride or nitrate by treatment with the appropriate concentrated mineral acid (90, 383).

$$\underset{\text{Nitrobiuret}}{NH_2CONHCONHNO_2} \rightarrow \underset{\text{Aminobiuret}}{NH_2CONHCONHNH_2} \underset{HCl}{\overset{C_6H_5CHO}{\rightleftharpoons}} NH_2CONHCONHN{=}CHC_6H_5$$

Benzalaminobiuret

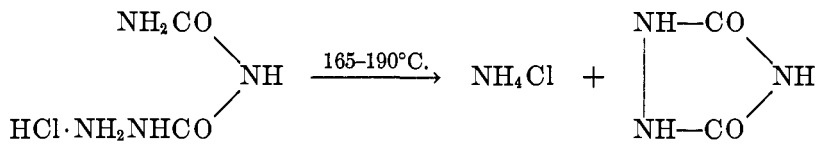
Aminobiuret is a monoacid base and forms a readily crystallizable hydrochloride, nitrate, and picrate. In common with primary amines, it yields solid condensation products with aldehydes (e.g., benzaldehyde) and ketones (e.g., acetone) (324, 383). Condensation with 5-nitrofurfural in aqueous acidic solution, for example, affords 95 per cent yields of 1-(5-nitrofurfurylideneamino)biuret (373).



5-Nitrofurfural + Aminobiuret →

1-(5-Nitrofurfurylideneamino)biuret

Pyrolysis of aminobiuret salts at 165–190°C. results in the elimination of an ammonium salt and formation of urazole (383).



Urazole

Owing to the presence of the —$NHNH_2$ group, aminobiuret shows certain analogies with semicarbazide. Thus, it is converted almost quantitatively to the corresponding azide (allophanic acid azide, $NH_2CONHCON_3$) by treatment with nitrous acid. Isopropylideneaminobiuret, the condensation product of biuret and acetone, is capable of adding hydrocyanic acid, to yield the appropriate nitrile (383).

$$\underset{\text{Isopropylideneaminobiuret}}{NH_2CONHCONHN{=}C(CH_3)_2} + HCN \rightarrow NH_2CONHCONHNHC(CH_3)_2CN$$

1-(Phenylamino)biuret is readily obtained by treatment of phenylhydrazine with allophanyl chloride. It is pyrolyzed, at 200–210°C., to ammonia and 1-phenylurazole (57, 58).

$$C_6H_5NHNH_2 + ClCONHCONH_2 \rightarrow C_6H_5NHNHCONHCONH_2 + HCl$$

Allophanyl chloride — 1-(Phenylamino)biuret

$$C_6H_5NHNHCONHCONH_2 \xrightarrow{200\text{–}210^\circ C.} C_6H_5N(NH)CO(CO)NH + NH_3$$

1-Phenylurazole

Finally, a series of substituted aminothiobiurets have been synthesized in good yields by condensing phenylhydrazine with carbamyl isothiocyanates, prepared *in situ* from disubstituted carbamyl chlorides and lead thiocyanate (99).

$$C_6H_5NHNH_2 + RR'NCONCS \rightarrow C_6H_5NHNHCSNHCONRR'$$

E. 1,5-DIAMINOBIURET

The reaction between ammoniatricarboxylic acid triethyl ester (*N*-tricarboxylic ester (XII)) and hydrazine does not yield the expected trihydrazide, but proceeds with simultaneous fission of the molecule and affords, in addition to ethyl hydrazinecarboxylate (XIII), appreciable quantities of imidodicarboxylic acid dihydrazide, i.e., 1,5-diaminobiuret (97). Detailed directions for the preparation of diaminobiuret by this reaction have been given in *Organic Syntheses* (6).

$$\underset{\text{XII}}{2N(COOC_2H_5)_3} + 5NH_2NH_2 + H_2O \rightarrow$$

$$\underset{\text{III}}{3NH_2NHCOOC_2H_5} + \underset{\text{1,5-Diaminobiuret}}{NH(CONHNH_2)_2} + 3C_2H_5OH + CO_2 + NH_3$$

Ethyl hydrazinecarboxylate

The product, which decomposes at 199–200°C., is highly soluble in water and readily crystallized from aqueous ethanol. It does not form salts, being readily cyclized by cold dilute acids, with loss of hydrazine, to urazole (97).

$$HN(CONHNH_2)_2 \rightarrow NH_2NH_2 + HN(CO{-}NH)(CO{-}NH)$$

1,5-Bis(diphenylamino)biuret was obtained by Michaelis (252) by the fusion of *as*-diphenylhydrazine and urea at 180°C. Since *as*-methylphenylhydrazine

gave *as*-methylphenylsemicarbazide under similar conditions, the production of biuret from diphenylhydrazine was considered to occur probably in the following stages:

$$NH_2CONH_2 + NH_2N(C_6H_5)_2 \rightarrow NH_3 + NH_2CONHN(C_6H_5)_2$$

XIV

$$(C_6H_5)_2NNHCONH_2 + NH_2CONHN(C_6H_5)_2 \rightarrow (C_6H_5)_2NNHCONHCONHN(C_6H_5)_2 + NH_3$$

1,5-Bis(diphenylamino)biuret

Pyrolysis of the postulated intermediate *as*-diphenylsemicarbazide (XIV) did indeed furnish the substituted diaminobiuret. The following alternative synthesis from *as*-diphenylhydrazine and biuret at 190°C. was held to provide further evidence for the suggested structure.

$$NH_2CONHCONH_2 + 2NH_2N(C_6H_5)_2 \xrightarrow{190°C.} 2NH_3 + (C_6H_5)_2NNHCONHCONHN(C_6H_5)_2$$

1,5-Bis(diphenylamino)biuret

The interaction of phenylbenzylsemicarbazide with phenylbenzylhydrazine under similar conditions gave a product which was regarded as 1,5-bis(benzylphenylamino)biuret (252).

1,5-Bis(phenylamino)biuret may be the minor product formed, together with diphenylcarbazide, when phenylhydrazine and 1,1-methylphenylurea are heated to 185–190°C. (214).

1,5-Diguanylbiuret

Acetylurea and guanidine carbonate react at 140–150°C. to yield 1,5-diguanylbiuret. In this reaction the deacetylation and condensation of two molecules of acetylurea may first give rise to biuret, which reacts subsequently with the guanidine to yield the observed product, $NH[CONHC(=NH)NH_2]_2$ (311). The interaction of fused urea and guanidine carbonate at 160–170°C. during 1–2 hr. leads to the same result (361). 1,5-Diguanylbiuret is an amorphous weak base; it is soluble in acids and alkalies, but may be precipitated from its acid solutions by means of ammonia. A crystalline nitrate has been obtained, but the salts are, in general, rapidly hydrolyzed (311).

The structurally related 1,5-dicaproylamidinobiuret,

$$NH[CONHC(=NH)C_5H_{11}]_2$$

has also been described (292).

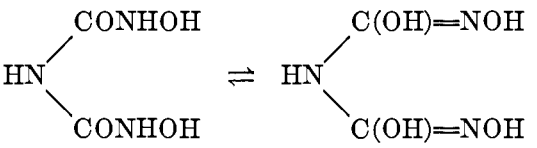
IV. Miscellaneous Biuret Derivatives

A. Oxybiuret and Dioxybiuret

The interaction of potassium cyanate and hydroxylamine salts, particularly the nitrate, at low temperatures yields oxyurea. In very dilute solution, under

carefully controlled conditions, the reaction affords a product of composition corresponding to oxybiuret. This compound might arise by the condensation of two molecules of cyanic acid and one of hydroxylamine, or by the elimination of hydroxylamine from two molecules of oxyurea. The structure of the product is not known with certainty; it may be 1- or 3-hydroxybiuret. Oxybiuret decomposes vigorously at its melting point, shows reducing properties, and yields oxyurea on treatment with dilute hydrochloric acid (103).

Mercury dimethyl and nitrogen peroxide in anhydrous ether at −15°C. yield a crystalline acidic compound of the formula $C_2H_5N_3O_4$, which may possibly have a dihydroxybiuret structure (XV); the remarkable instability of the product, which can rarely be stored for longer than 30 min., however, has prevented its detailed examination (24).



XV

Phenyl isocyanate and hydroxylamine condense analogously to yield, according to the proportions of the reactants employed, 3-hydroxy-1-phenylurea or 3-hydroxy-1,5-diphenylbiuret. Moreover, the former product is convertible to the latter by treatment with more isocyanate (119, 217, 220).

$$C_6H_5NCO + NH_2OH \rightarrow C_6H_5NHCONHOH$$

3-Hydroxy-1-phenylurea

$$2C_6H_5NCO + NH_2OH \searrow (C_6H_5NHCO)_2NOH$$

$$C_6H_5NCO + C_6H_5NHCONHOH \nearrow$$

3-Hydroxy-1,5-diphenylbiuret

N-Alkylhydroxylamines are capable of reacting with only *one* molecule of isocyanate (220). Treatment of 3-hydroxy-1-phenylurea with hydrochloric acid (217) or with boiling alcohol (220) also yields 3-hydroxy-1,5-diphenylbiuret. This substituted hydroxybiuret is insoluble in alkalies, and, unlike 3-hydroxy-1-phenylurea, fails to reduce Fehling's solution (119, 217). It is decomposed into aniline, carbanilide, hydroxylamine, and carbon dioxide by boiling alkalies (220).

An alternative formulation of oxybiuret and its diphenyl derivative as hydroxamic acid derivatives

$$NH_2CONHOCONH_2 \quad \text{and} \quad C_6H_5NHCONHOCONHC_6H_5$$

has been proposed (199), but the problem has yet to be fully resolved.

B. HALOGENATED BIURETS

A halogenated derivative, probably 1,5-dichlorobiuret, has been prepared by chlorinating biuret in aqueous solution. The crystallized product is fairly stable and decomposes only slowly on prolonged storage (88).

Aliphatic aldehydes react with freshly prepared aqueous chlorourea to yield a variety of halogen-containing products which are believed to conform to different classes of structural patterns, depending on the aldehyde selected. Propionaldehyde yields an insoluble amorphous solid which is regarded, on the basis of its composition, as 5-chloro-1-propylidenebiuret ($C_2H_5CH{=}NCONH$-$CONHCl$), but further experimental evidence in support of this formulation is required (278).

In common with analogous *N*-chloro derivatives, 1,5-dichlorobiuret has been claimed to render woolen material resistant to felting shrinkage (108).

C. CYANOBIURETS

In ethereal solution, at low temperatures, methyl isocyanate and cyanamide condense, in the presence of triethylphosphine, to yield 3-cyano-1,5-dimethylbiuret (358).

All reactions of this labile compound appear to involve its primary fission into 3-cyano-1-methylurea and methyl isocyanate; the former may in fact be isolated almost quantitatively on treatment of 3-cyano-1,5-dimethylbiuret with hydrogen sulfide. The use of an excess of this reagent affords 1-methyl-4-thiobiuret as expected. Several analogous reactions of the cyanobiuret are clearly those of 3-cyano-1-methylurea, formed as the primary intermediate in all cases (358).

$$2CH_3NCO + NH_2CN \xrightarrow{P(C_2H_5)_3} (CH_3NHCO)_2NCN$$

3-Cyano-1,5-dimethylbiuret

$$\downarrow$$

$$CH_3NHCONHCSNH_2 \xleftarrow{H_2S} CH_3NHCONHCN + CH_3NCO$$

1-Methyl-4-thiobiuret — 3-Cyano-1-methylurea

D. BIURET ANILS

A number of Schiff bases derived from biuret, e.g., benzylidenebiuret (332), have been studied. They are sometimes obtained, though only in moderate yields, when aldehydes or ketones react with urea at elevated temperatures. Prolonged interaction of 5-chloro- or 3,5-dichlorosalicylic aldehyde and urea at 140–150°C., for example, yields salicylidenebiuret (263). At 170°C. acetophenone and propiophenone are converted into condensation products of the structure $(C_6H_5AlkC{=}N)_2CO$. Benzophenone, however, affords small proportions of the product $(C_6H_5)_2C{=}NCONHCONH_2$. The reaction may involve the elimination of ammonia between benzophenylideneurea ($(C_6H_5)_2C{=}NCONH_2$) and urea (342). Support for this view appears to be provided by the conversion of benzylideneurea into benzylidenebiuret in hot nitrobenzene (438).

V. Substituted Biurets

This section deals with alkyl- and arylbiurets. For easy reference, derivatives have been classified according to the number and position of their substituents. Repetitions which are liable to be introduced by this treatment have been avoided as far as possible by the use of numerous cross references.

A. 1-Substituted Biurets

1. Synthesis

(a) From allophanic esters

The synthesis of biuret from allophanic ester and ammonia (*cf.* Section II,B,4) is readily extended to the production of substituted biurets. Treatment of methyl allophanate with aqueous methylamine or ethylamine, for example, in closed vessels at 100°C. affords 1-alkylbiurets readily in good yields (40, 193, 261, 290). Equimolecular quantities of methyl allophanate and aniline or *o*-toluidine react at 140–170°C. to yield 1-arylbiurets (together with 1,5-diarylbiurets and other products) (84).

$$NH_2CONHCOOCH_3 + NH_2R \xrightarrow{140\text{–}170°C.} NH_2CONHCONHR + CH_3OH$$

Methyl allophanate

The carbethoxy group of ethyl allophanate, however, remains unaffected under similar conditions, ethyl 4-arylallophanate being formed instead (84).

$$NH_2CONHCOOC_2H_5 + NH_2R \xrightarrow{140\text{–}170°C.} RNHCONHCOOC_2H_5 + NH_3$$

Ethyl allophanate

The interaction of 4-alkyl- or 4-arylallophanic esters with ammonia constitutes a valuable and widely used variation of this synthesis (40, 66, 67, 111, 155, 427).

$$RNHCONHCOOC_2H_5 + NH_3 \rightarrow RNHCONHCONH_2 + C_2H_5OH$$

Ethylenebisbiuret, $[—CH_2NHCONHCONH_2]_2$, has been prepared from ethylenebis(carbethoxyurea) by this method (101).

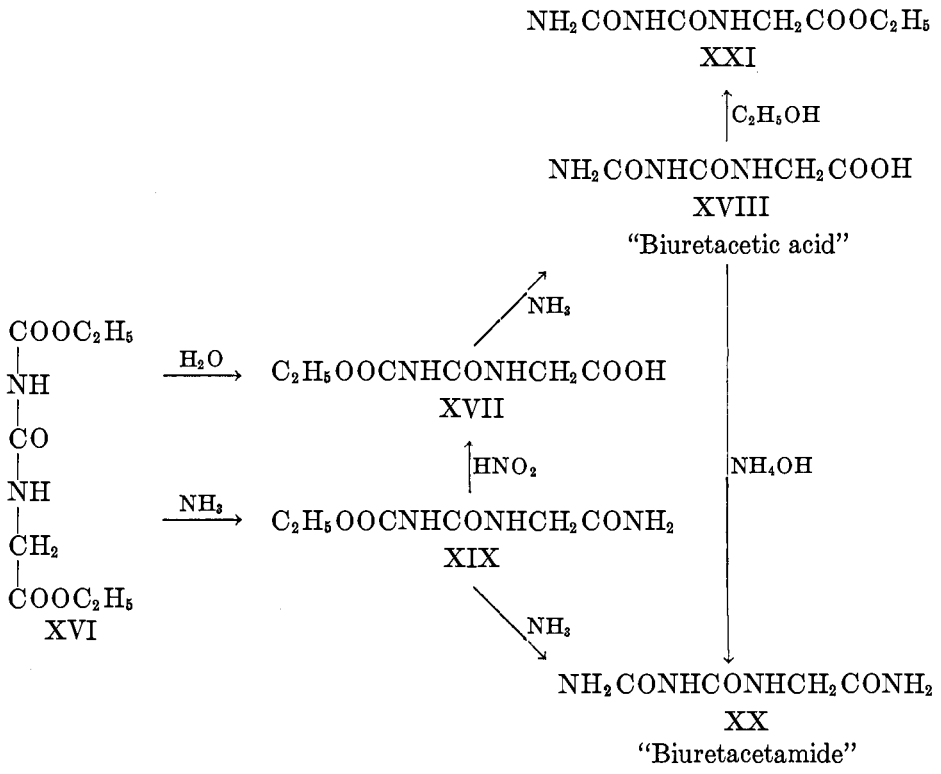
"Biuretacetic acid" (XVIII), its ethyl ester, and its amide have been synthesized from 1-carbethoxy-3-methylcarbethoxyurea (XVI), which is obtainable in small yields from ethyl chloroacetate, potassium cyanate, and ethanol.

$$CH_2ClCOOC_2H_5 + 2KCNO + C_2H_5OH + H_2O \rightarrow KCl + KOH + C_2H_5OOCNHCONHCH_2COOC_2H_5$$

XVI

Of the two carbethoxy groups in XVI, the one attached to the methylene carbon atom is more reactive; successive hydrolysis and ammonolysis yield "biuretacetic acid" (XVI → XVII → XVIII), while direct ammonolysis produces "biuretacetamide" (XVI → XIX → XX). The latter is also formed by the

pyrolysis of the ammonium salt of biuretacetic acid (XVIII → XX). The acid (XVIII) may be converted to an ester (XXI) by the usual methods (143).



The reported preparation of biuretacetic acid derivatives (110) from carbethoxyureidomethane ($NH_2CONHCH_2COOC_2H_5$) has not been confirmed, the products being subsequently identified as impure starting materials (143).

(b) From allophanyl chloride

The interaction of allophanyl chloride and primary amines affords a similar route to 1-substituted biurets and provides, at the same time, confirmation of the structure of these products. The reaction occurs readily in dry benzene and has been successfully performed with aliphatic, alicyclic, and aromatic amines (57, 58).

$$NH_2CONHCOCl + NH_2R \rightarrow RNHCONHCONH_2 + HCl$$

Allophanyl chloride

(c) Hydration of cyanoureas

1-Substituted biurets are accessible by the addition of the elements of water to 1-substituted-3-cyanoureas. The hydration may be effected by an excess of hydrogen peroxide in alkaline solution (52), by boiling dilute sulfuric acid (150),

or by warm concentrated sulfuric acid, followed by immediate dilution with ice (52).

$$RNHCONHCN + H_2O \rightarrow RNHCONHCONH_2$$

1,1-Diphenylbiuret has been obtained by a modification of this synthesis (235).

(d) From isocyanates and urea

The condensation of isocyanates and urea at elevated temperatures affords alkylbiurets readily in good yields (40).

$$RNCO + NH_2CONH_2 \rightarrow RNHCONHCONH_2$$

Phenyl isocyanate and urea, heated at 100°C. for 7 hr., yield 1-phenylbiuret as the main product; at higher temperatures complex mixtures of phenylurea, carbanilide, and condensation products of urea, ammonia, and isocyanic acid are formed (236).

(e) From azides

Oxamic acid azide[2] results from the action of sodium nitrite on aqueous oxamic acid hydrazide hydrochloride at −5°C. and is separated from dioxamidohydrazine, $[NH_2COCONH—]_2$, formed as a by-product, by its solubility in cold acetone. In reacting with primary aromatic amines, it undergoes the Curtius rearrangement and yields 1-arylbiurets, with simultaneous loss of nitrogen (79; see also 12).

$$NH_2COCON_3 \rightarrow [N_2 + NH_2CONCO] \xrightarrow{RNH_2} RNHCONHCONH_2$$

1,5-Disubstituted biurets are similarly formed: the interaction of benzyloxamic acid azide and boiling *p*-toluidine, for example, affords, in addition to much benzyl-*p*-tolyloxamide ($C_7H_7NHCOCONHC_6H_4CH_3$), small yields of 1-benzyl-5-*p*-tolylbiuret (80). In a recent application of this method, Sah and his coworkers (325) condensed the azides of four aryloxamic acids ($ArNHCOCON_3$: Ar = C_6H_5, *p*-$CH_3C_6H_4$, 1-$C_{10}H_7$, 2-$C_{10}H_7$) with a large variety of amines in boiling anhydrous benzene or toluene. The resulting series of one hundred and twenty 1,5-disubstituted biurets consisted of well-defined easily crystallizable solids, which are claimed to be suitable derivatives for the identification of amines.

1,1,5-Trisubstituted biurets are similarly obtained from diphenylamine or piperidine (12).

(f) From hydroxyoxamides

Alkali salts of acylhydroxamic acids are readily hydrolyzed to isocyanates, which are convertible, *in situ*, to monosubstituted ureas, disubstituted ureas, or urethans by means of ammonia, water, or an alcohol, respectively (382).

$$\begin{matrix} & OK \\ & | \\ RC & =NOCOCH_3 \end{matrix} \rightarrow CH_3COOK + RNCO \rightarrow \text{products}$$

[2] Oxamic acid azide detonates when crushed and explodes violently at 115°C.

The extension of this reaction to the analogous monohydroxyoxamides (XXII) affords a convenient method of preparing biurets, as well as allophanates and triurets (*cf.* Section XIII).

$$\underset{\text{XXII}}{\text{RNHCOC(OK)=NOCOCH}_3} \rightarrow \text{CH}_3\text{COOK} + [\text{RNHCONCO}]$$

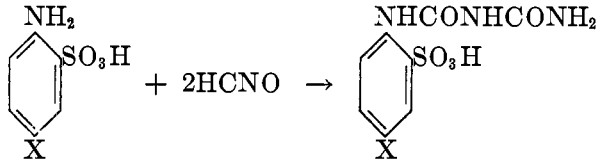
$$[\text{RNHCONCO}] + \text{NH}_3 \rightarrow \text{RNHCONHCONH}_2$$

Monohydroxyoxamides (RNHCOCONHOH) are obtained quantitatively by hydrolyzing ethyl oxamates ($\text{RNHCOCOOC}_2\text{H}_5$) with anhydrous alcoholic solutions of hydroxylamine. Their readily accessible acetyl derivatives (XXII) form well-defined crystalline metallic salts, which decompose in boiling dilute ammonia, often with violence, to monosubstituted biurets. The method is applicable to aliphatic and aromatic members of this series (289, 290), but varying quantities of by-products are liable to be formed. Thus, while preparing 1-phenylbiuret by this synthesis, Schiff (335) isolated 1,5-diphenylbiuret, ammonium oxanilate, and oxanilide.

(g) From cyanic acid and amines

Small quantities of substituted biurets are occasionally formed as by-products in the preparation of ureas from amines and cyanic acid (91, 98, 162, 229). This result is analogous to Drechsel's synthesis (102) of biuret from urea and potassium cyanate in acetic acid solution. Davis and Blanchard (91) have adduced evidence that the preliminary dimerization of small proportions of cyanic acid to dicyanic acid accounts for the formation of biuret. Secondary amines similarly yield small amounts of 1,1-disubstituted biurets (e.g., 1-methyl-1-phenyl and 1-methyl-1-*p*-tolyl homologs) (381).

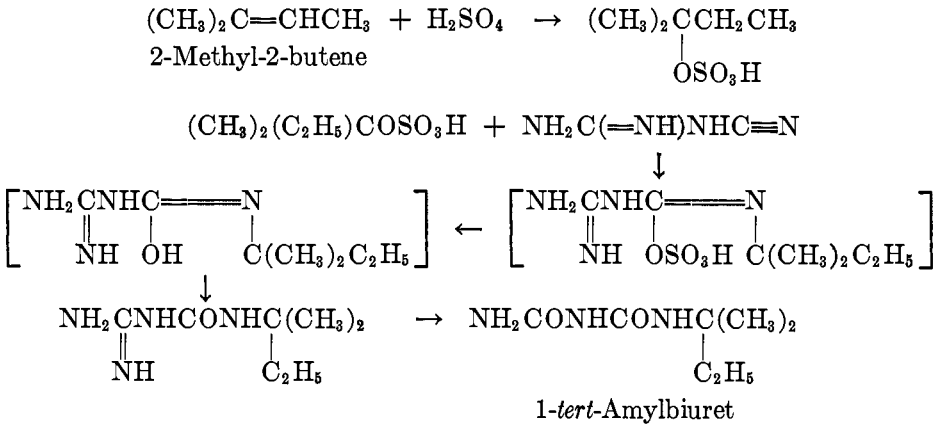
It is remarkable that substituted biurets, and not ureas, are the main product of the interaction of 2-amino-5-halogenobenzenesulfonic acids with cyanic acid (346).



(h) From dicyandiamide and alkenes

The condensation of alkenes and nitriles, in the presence of concentrated sulfuric acid, has recently been found to yield amides. In an extension of this general synthesis 1-*tert*-amyl- and 1-*tert*-octylbiuret were obtained by the condensation of dicyandiamide with 2-methyl-2-butene or diisobutylene, respectively. On the basis of the authors' general mechanism of this reaction (318),

the formation of biurets may be interpreted as follows (317, 318):



(i) From nitrobiuret

The formation of 1-substituted biurets from nitrobiuret and primary amines occurs readily when equivalent quantities of the reactants are warmed, in aqueous solution, until evolution of nitrous oxide ceases. The resulting biuret is separated from small quantities of cyanuric acid by extraction with organic solvents, in which this by-product is insoluble (90). The method has been extended to the preparation of heterocyclic derivatives, including 1-(2-pyridyl)- and 1-(2-thiazolyl)biurets, in 34 and 13 per cent yields, respectively (94); furfurylamine, however, yields mainly furfurylurea (353).

$$RNH_2 + NH_2CONHCONHNO_2 \rightarrow RNHCONHCONH_2 + N_2O + H_2O$$

(j) From thiobiurets

The desulfurization of suitable thiobiurets is a general method of obtaining biurets. 1-Phenylbiuret, for example, results from 1-phenyl-4-thiobiuret on treatment with lead hydroxide in alcohol (236).

(k) From 1,4-disubstituted-4-isobiurets (*cf.* Section VI,B)

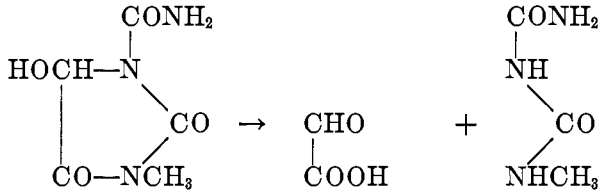
(l) From triuret derivatives

Small yields (up to 10 per cent) of 1-methylbiuret are obtained, in addition to much *N*-methylcyanuric acid, when 1,7-dimethyl-1-nitrosotriuret is warmed with water (121; compare also 40).

(m) From heterocyclic compounds, including purines

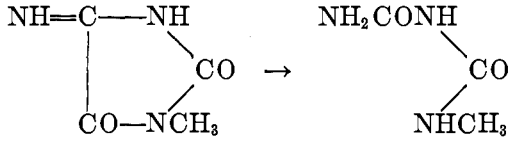
Homologs of biuret are occasionally isolated amongst the degradation products of purines. Hydrolysis by barium hydroxide of 1-carbonamido-5-hydroxy-3-methylhydantoin (a product of the oxidative degradation of purines) splits

the heterocyclic nucleus almost quantitatively into glyoxylic acid and 1-methylbiuret (41).



Glyoxylic acid 1-Methylbiuret

Acid hydrolysis of 3-methylallantoxaidine similarly yields 1-methylbiuret (38).



In the oxidation of 9-phenyluric acid by hydrogen peroxide, Moore and Gatewood (154, 256) obtained 1-phenylbiuret and a "third phenylbiuret, m.p. 196–198°" (differing in properties from both 1- and 3-phenylbiuret then known in the literature), which was convertible into 1-phenylbiuret by bases. Blair (50) identified this product as an addition compound of 3 moles of 1-phenylbiuret and 2 moles of phenylisocyanuric acid. Its supposed "isomerization" to 1-phenylbiuret is simply due to the greater increase in solubility of the more strongly acidic phenylisocyanuric acid in the presence of bases; this component is thus held in solution, while pure 1-phenylbiuret crystallizes out.

2. Properties

1-Substituted biurets act as weak acids; they are generally dissolved by dilute alkalies and reprecipitated by mineral acids (58, 154). The lower homologs are readily soluble in boiling water, acetic acid, and most organic solvents (41). Some crystallographic and optical properties of 1-phenylbiuret have been recorded (154, 155).

Substituted biurets decompose at or above their melting points, and special precautions are necessary to ensure the reproducibility of the results of melting-point determinations. The decomposition point of 1-phenylbiuret, for example, depends on the rate of heating, and has variously been given between 156°C. and 171°C. (50, 235). During this pyrolysis (usually in the temperature range of 200–260°C.) 1-arylbiurets are split into ammonia, cyanic acid (and thence cyanuric acid), and the appropriate carbanilide; 1-alkylbiurets decompose analogously, but yield a monoalkylurea as final decomposition product (57, 58).

$$2RNHCONHCONH_2 \rightarrow RNHCONHR + NH_3 + C_3H_3N_3O_3$$

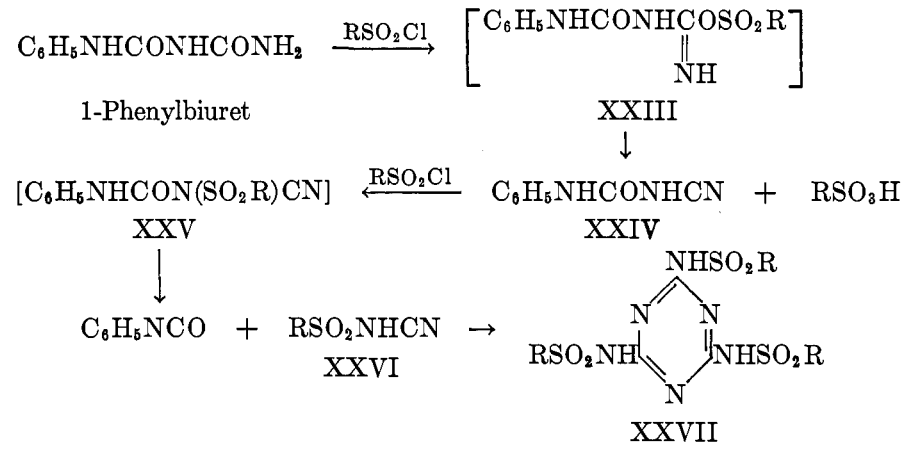
A molecular compound, m.p. 197–198°C., consisting of 3 moles of 1-phenylbiuret and 2 moles of phenylisocyanuric acid, forms a continuous series of solid

solutions with each component (50). This product, obtained in the oxidation of 9-phenyluric acid, had first been erroneously regarded as 3-phenylbiuret (154, 256).

The action of nitrous acid upon 1-alkylbiurets affords yellow nitroso derivatives (*cf.* Section III,C). On similar treatment 1-phenylbiuret does not evolve nitrogen, but gives pale yellow to pink solutions on subsequent addition of alkaline β-naphthol (346).

Alkylation of 1-phenylbiuret by dimethyl sulfate yields a monomethyl derivative, which differs from authentic 1,5-methylphenylbiuret and has therefore been formulated as the 1,3-disubstituted product (155).

In common with aromatic ureas and thioureas (228), 1-arylbiurets do not yield *N*-sulfonyl derivatives on treatment with sulfonyl chlorides: in pyridine solution, isocyanates and trisulfonylmelamines (XXVII) are the main products of this reaction. 1-Phenylbiuret reacts with an excess of *p*-toluenesulfonyl chloride, for example, to give 1,3-diphenylurea (from the primary product, phenyl isocyanate) and tri(*p*-toluenesulfonyl)melamine, in 60 and 45 per cent yields, respectively (233, 235). These changes have been accounted for by a mechanism which correlates the results satisfactorily with those in the urea series (228).



The first stage in this sequence is the formation of labile 1-aryl-4-sulfonyl-4-isobiurets (XXIII). Like other imidosulfonates (228, 279, 280), these decompose spontaneously with elimination of sulfonic acids to yield, in the present case, 1-aryl-3-cyanoureas. Further interaction with the excess of sulfonyl halide probably leads to 1-aryl-3-cyano-3-sulfonylureas (XXV), which break down into aryl isocyanates and arylsulfonylcyanamides (XXVI). The former are converted, in the presence of water, to carbanilides, while the latter polymerize to trisulfonylmelamines (XXVII) and other, unidentified polymers. The correctness of the suggested mechanism is supported by the observation that 1-aryl-3-cyanoureas (XXIV), postulated as intermediates in this reaction, react with aromatic sulfonyl chlorides even more smoothly, with identical results (235).

In contrast to isourea ethers, which yield *N*-sulfonyl derivatives readily (228), the structurally related 4-methyl-1-phenyl-4-isobiuret fails to react with sulfonyl chlorides in alkaline media. In pyridine, small yields of trisulfonylmelamines (XXVII) are produced (235), probably by previous demethylation of the isobiuret by the pyridinium chloride formed *in situ* (*cf.* 230), followed by the usual reaction sequence (XXIII → XXVII).

B. 3-SUBSTITUTED BIURETS

1. Synthesis

(a) From 1-arylureas

When treating phenylurea with phosphorus trichloride, Weith (419) obtained a phenylbiuret which was later identified by Schiff (335) as the 3-substituted isomer, because of its strongly positive biuret reaction and its easy conversion into 1,3-diphenylurea on treatment with aniline (1-phenylbiuret yields 1,5-diphenylbiuret under these conditions (335)). The reaction affords only poor yields, however; in spite of Schiff's improvements, later workers experienced great difficulties (154) or failed entirely (52, 346) to repeat this work.

(b) From 2-alkylallophanates

3-Substituted biurets are available by the ammonolysis of 2-alkylallophanates, obtained in turn from urethans and carbamyl chloride (40).

$$NH_2COCl + RNHCOOCH_3 \rightarrow NH_2CONRCOOCH_3$$

$$NH_2CONRCOOCH_3 + NH_3 \rightarrow NH_2CONRCONH_2 + CH_3OH$$

Good yields of 3-methylbiuret are obtained, for example, by treating methyl 2-methylallophanate, in closed vessels, with concentrated ammonia at 100°C. The synthesis of the 3-ethyl homolog is complicated by the predominating hydrolysis of the starting material to ammonium 2-ethylallophanate. In the aromatic series, the reported synthesis of 3-phenylbiuret (40) could not be repeated by another investigator (155). The general method fails to afford the expected 1,3-disubstituted biurets from primary amines.

Abortive attempts to obtain 3-phenylbiuret by the hydrolysis of 1-cyano-1-phenylurea (346) or by the ammonolysis of aniline-*N*-dicarboxylic ester [$C_6H_5N(COOC_2H_5)_2$] (154) are on record.

2. Properties

It is noteworthy that the melting points of 3-substituted biurets do not differ significantly from the decomposition point of biuret (190–191°C.), while those of the corresponding 1-substituted products differ considerably (40) (*cf.* table 4).

Some crystallographic and optical properties of 3-phenylbiuret have been described (154, 155). 3-Substituted biurets give a strong biuret reaction (40, 335).

TABLE 4

Melting points of monosubstituted biurets

Biuret	Melting Point	Biuret	Melting Point
	°C.		°C.
3-Methylbiuret	189	1-Methylbiuret	168
3-Ethylbiuret	179	1-Ethylbiuret	154
3-Phenylbiuret	192	1-Phenylbiuret	172

C. 1,1-DISUBSTITUTED BIURETS

1. Synthesis

(a) From allophanic esters

Allophanates react with secondary amines in the expected manner (*cf.* Section V,A,1(a)): methyl allophanate and aqueous dimethylamine in closed vessels at 100°C., for example, form 1,1-dimethylbiuret (40).

$$NH_2CONHCOOCH_3 + R'R''NH \rightarrow R'R''NCONHCONH_2 + CH_3OH$$

However, attempts to synthesize a trisubstituted biuret from 4-methylallophanic ester by this method were unsuccessful (40).

(b) From allophanyl chloride

The interaction of allophanyl chloride and secondary amines similarly yields 1,1-disubstituted biurets (58) (*cf.* Section V,A,1(b)).

(c) From nitrobiuret

The reaction of nitrobiuret with secondary amines, in the presence of water, affords good yields of 1,1-disubstituted biurets (90) (*cf.* Section V,A,1(i)).

$$NH_2CONHCONHNO_2 + R'R''NH \rightarrow R'R''NCONHCONH_2 + H_2O + N_2O$$

Nitrobiuret

(d) From nitrourea

In certain cases small quantities of 1,1-disubstituted biurets have been observed as by-products in the synthesis of substituted ureas from amines and nitrourea. Thus, di-*n*-propylamine and *n*-propylaniline give rise to small amounts of biurets, in addition to the appropriate ureas (89).

(e) From cyanic acid and amines (*cf.* Section V,A,1(g))

(f) Hydration of cyanoureas (*cf.* Section V,A,1(c))

D. 1,3-DISUBSTITUTED BIURETS

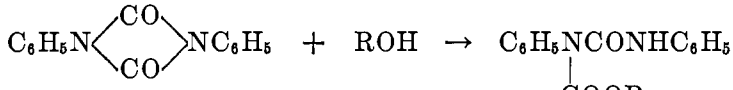
1. Synthesis

(a) Alkylation of 1-arylbiurets

The methylation, by means of dimethyl sulfate, of 1-phenylbiuret yields a product which is regarded as the 1,3-isomer, because of its non-identity with authentic 1,5-phenylmethylbiuret (155).

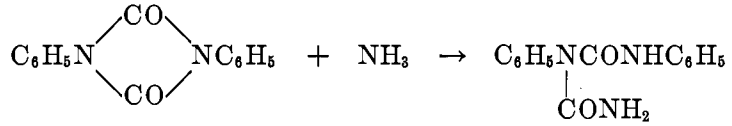
(b) From diaryl diisocyanates ("uretediones")

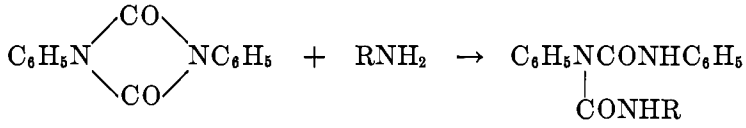
Hofmann (192) first observed in 1871 that phenyl isocyanate polymerizes, under the influence of triethylphosphine, to a dimeric product. The structure (XXVIII)[3] of the dimer follows from its conversion, by alcohols, into alkyl 2,4-diphenylallophanates (192; see also 369).



XXVIII

By an analogous mechanism, diphenyl diisocyanate reacts with ammonia to yield 1,3-diphenylbiuret (192). The reaction has been employed for preparing 1,3-dialkylbiurets (93, 357) and is, of course, applicable to the preparation of more highly substituted homologs by the use of amines in place of ammonia (310). Thus, 1,3,5-triphenylbiuret, the first symmetrically trisubstituted biuret, was obtained by Hofmann in 1871 by this method (192).





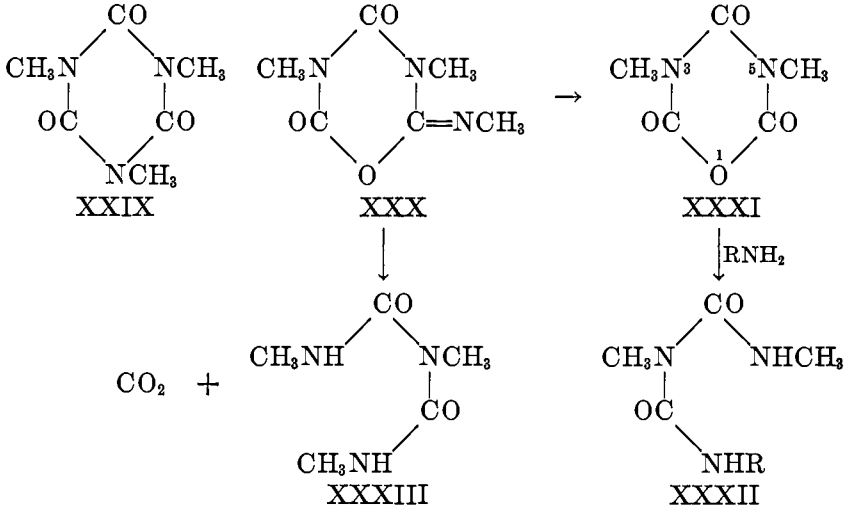
(c) From 1,3,5-oxdiazines

The polymerization of methyl isocyanate, normally yielding trimethyl isocyanurate (XXIX), proceeds differently at low temperatures (−80°C.) in the presence of triethylphosphine and affords a mixture of 3,5-dimethyl-2-methylimino-4,6-diketo-1,3,5-oxdiazine (XXX) and 3,5-dimethyl-2,4,6-triketo-1,3,5-oxdiazine (XXXI). The latter compound (XXXI) (which may be regarded as made up of two molecules of methyl isocyanate and one molecule of carbon dioxide) becomes the exclusive product when solid carbon dioxide is introduced

[3] For an alternative formulation of dimeric isocyanates, *cf.* the proposals of Gaylord and Crowdle (160).

during the polymerization and is, moreover, obtainable by the acid hydrolysis of XXX.

3,5-Dimethyl-2,4,6-triketo-1,3,5-oxdiazine (XXXI), on treatment with ammonia or aliphatic (but not aromatic) amines, is converted, with loss of carbon dioxide, to 1,3-dialkylbiurets or 1,3,5-trialkylbiurets (XXXII), respectively. In an analogous ring fission, XXX yields 1,3,5-trimethylbiuret (XXXIII), simply on being boiled with water (357).



2. Properties

1,3-Diphenylbiuret is decomposed into phenyl isocyanate and ammonia by hydrogen chloride (193).

E. 1,5-DISUBSTITUTED BIURETS

1. Synthesis

(a) From allophanic esters

When treating ethyl allophanate with aniline at the boiling point, Hofmann (193) did not obtain the expected phenylbiuret: the reaction proceeded, with elimination of ammonia, to the 1,5-diphenylbiuret stage. The first step is probably the formation of ethyl 4-phenylallophanate; this is in fact isolated when equimolecular proportions of the reactants are employed. In the case of methyl allophanate, however, 1-arylbiurets arise as intermediates because of the preferential elimination of the methoxy group (84).

$$NH_2CONHCOOC_2H_5 + C_6H_5NH_2 \rightarrow C_6H_5NHCONHCOOC_2H_5 + NH_3$$

Ethyl allophanate — Ethyl 4-phenylallophanate

$$C_6H_5NHCONHCOOC_2H_5 + C_6H_5NH_2 \rightarrow (C_6H_5NHCO)_2NH + C_2H_5OH$$

1,5-Diphenylbiuret

$$NH_2CONHCOOCH_3 + C_6H_5NH_2 \rightarrow NH_2CONHCONHC_6H_5 + CH_3OH$$

Methyl allophanate — 1-Phenylbiuret

A number of allophanates (84, 261), including thio analogs ($NH_2CONHCOSC_2H_5$, $CH_3NHCONHCOSCH_3$) (114, 283), have been used with success in this synthesis. As expected, 4-alkyl(or aryl)allophanates and primary amines yield 1,5-disubstituted biurets directly (40, 76, 155), but attempts to employ methyl 4-phenyl-3-thioallophanate were unsuccessful (99).

The interaction of 1,3-dicarbethoxyurea and amines may be regarded as an extension of this synthesis. In the temperature range of 110–170°C. varying quantities of 1,5-diarylbiurets are formed, together with arylcarbethoxybiurets (*cf.* Section XII,B) and 1,7-diaryltriurets (*cf.* Section XIII,B). The usefulness of the method is limited because of the difficulties of separating the individual products (82, 261).

(b) From 1-substituted ureas and isocyanates

1,5-Diarylbiurets have been prepared by the condensation of aromatic ureas and isocyanates at 120–150°C. (227); at lower temperatures some monosubstituted biuret is also formed (236).

The course of this reaction is liable to be somewhat erratic, particularly at temperatures exceeding the melting point of the urea. Thus phenyl isocyanate condenses, according to the conditions, with either the amino or the methylamino group of 1-methylurea, forming 5-methyl-1-phenyl- and 3-methyl-1-phenylbiuret, respectively (35, 155; see also 76). In some cases the presence of large quantities of by-products renders the isolation of the desired compounds impossible; the condensation of phenyl isocyanate and *p*-chlorophenylurea, for example, gave unseparable mixtures (76).

A number of aliphatic homologs have been synthesized by this method (40). Ethylurea and ethyl isocyanate at 100°C., however, do not yield the expected 1,5-diethylbiuret; a product of unknown constitution, formed by further loss of water, is isolated instead (40).

(c) From azides (*cf.* Section V,A,1(e))

(d) From biuret

Hofmann showed, in 1871, that aromatic substituents are conveniently introduced into the biuret structure by the direct action of arylamines on biuret. A boiling solution of the parent compound in aniline affords good yields of 1,5-diphenylbiuret (193), as does 1-phenylbiuret at lower temperatures; with boiling aniline, however, 1,3-diphenylurea becomes the main product (335; see also 346).

$$NH_2CONHCONH_2 + 2RNH_2 \rightarrow (RNHCO)_2NH + 2NH_3$$

Fosse's well-known synthesis of dixanthydrylurea, which is used in the quantitative estimation of urea, has its parallel in the biuret series: 1,5-dixanthydrylbiuret is formed in the expected manner from xanthydrol and biuret (125).

(e) From 1,2,5-trisubstituted-2-isobiurets (*cf.* Section VI,C)

(f) Miscellaneous reactions

Prolonged treatment of phenylurea with phosgene in toluene at 100°C. does not result in the expected triuret, but affords 1,5-diphenylbiuret instead (335). This compound is also precipitated slowly from aqueous solution of the ammonium salt of *N*-chlorobenzamide, $C_6H_5CONClNH_4$ (109).

The replacement of one substituent by another in 1,5-disubstituted biurets has been observed: 1-methyl-5-phenylbiuret, on treatment with aniline just below its boiling point, is converted into 1,5-diphenylbiuret (155). Finally, small yields of the symmetrically disubstituted urea and biuret are formed in the interaction of *p*-ethoxyphenylurea and ethyl chloroformate at 175°C. (427).

2. Properties

1,5-Diphenylbiuret is unaffected by nitrous acid (346). It is decomposed by hydrogen chloride into phenyl isocyanate and aniline (193). Some crystallographic and optical properties of 1-methyl-5-phenylbiuret are on record (155).

F. 1,1,5-TRISUBSTITUTED BIURETS

A number of 1,1,5-trisubstituted biurets have been obtained by the general syntheses already discussed, appropriately substituted starting materials being chosen. These methods include the interaction of azides with secondary amines (*cf.* Section V,A,1(e)), the demethylation of 1,2,5,5-tetrasubstituted-2-isobiurets (*cf.* Section VI,D), and the desulfurization of corresponding thiobiurets (e.g., the 1,1-diphenyl-5-*o*-tolyl-4-thio homolog) (99).

Like nitrobiuret, 1,1-dimethyl-5-nitrobiuret reacts with amines in aqueous solution, to furnish trisubstituted biurets (92).

$$(CH_3)_2NCONHCONHNO_2 + RNH_2 \rightarrow$$

1,1-Dimethyl-5-nitrobiuret

$$(CH_3)_2NCONHCONHR + H_2O + N_2O$$

G. 1,3,5-TRISUBSTITUTED BIURETS

1. Synthesis

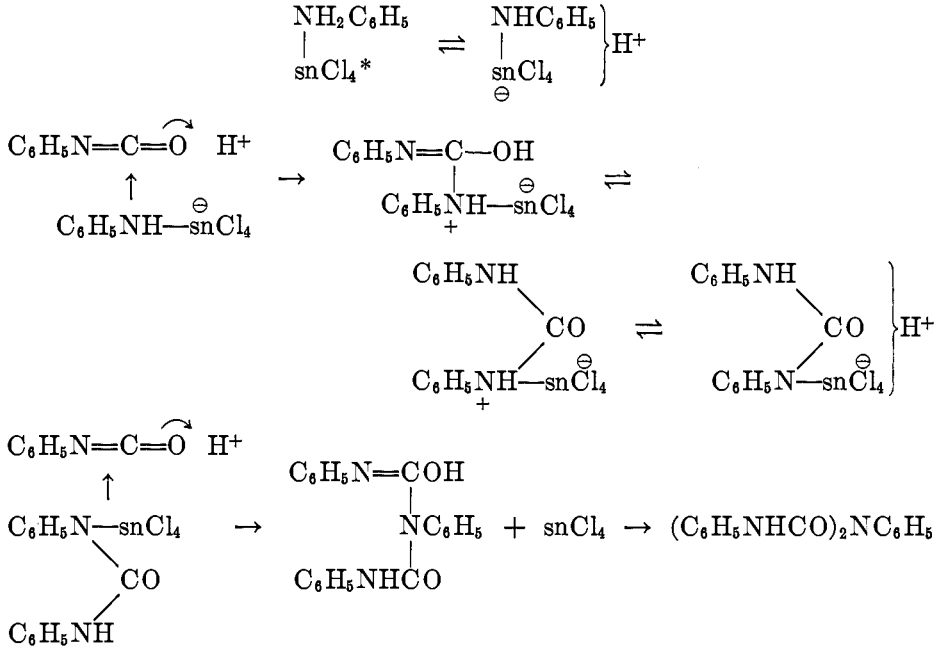
(a) From 1,3-disubstituted ureas and isocyanates

The condensation of substituted ureas and isocyanates has been successfully applied to the preparation of 1,3,5-trisubstituted biurets (227, 312). At 150°C. carbanilide and phenyl isocyanate, for example, yield the 1,3,5-triphenyl derivative. The reaction is reversible, however, the product decomposing above its melting point (147°C.) into the starting materials (227; see also 40, 236).

$$RNHCONHR' + R''NCO \rightarrow RNHCONR'CONHR''$$

Further details concerning this reaction have recently been provided by the work of Baker and Holdsworth (20). Carbanilide and phenyl isocyanate do not combine at room temperature, but do so readily in the presence of anhydrous stannic chloride. The reaction appears to be accelerated by the formation of intermediate addition complexes; separate experiments reveal that both aniline

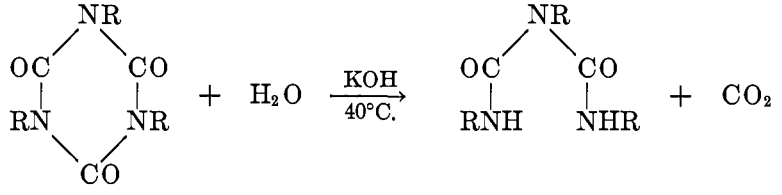
and carbanilide form addition compounds with stannic chloride ($2C_6H_5NH_2 \cdot SnCl_4$ and $3[CO(NHC_6H_5)_2] \cdot 2SnCl_4$, respectively) which show a greatly increased reactivity towards phenyl isocyanate. Both complexes afford 1,3,5-triphenylbiuret at room temperature in excellent yields. The observations are in agreement with the following suggested mechanism for this catalyzed reaction (20):



* The symbol $snCl_4$ represents that proportion of the $SnCl_4$ molecule which is stoichiometrically associated with *one* molecule of the other component.

(b) From trialkylisocyanuric acids

The alkaline hydrolysis of *N*,*N*,*N*-trialkylisocyanuric acids, first observed by Habich and Limpricht (171, 241) in 1858 and correctly interpreted by Nencki nearly twenty years later (262), affords symmetrically trisubstituted biurets. Boiling barium hydroxide or 1 *N* potassium hydroxide at 40°C. readily effects this reaction (120).



(c) From diaryl diisocyanates (uretediones) (*cf.* Section V,D,1(b))

(d) From 1,3,5-oxdiazines (*cf.* Section V,D,1(c))

(e) From olefins and aryl isocyanates

Under the catalytic influence of stannic chloride, olefins of the structure $CH_3CR{=}CR_2$ (R = H or alkyl) change to polymers $(CH_2)_n$ of varying complexity; in the presence of phenyl isocyanate this main polymerization is accompanied by another, probably much slower, reaction, which terminates with the production of small yields of 1,3,5-triphenylbiuret (20).

Thus, a number of suitable olefins (including propylene, trimethylethylene, tetramethylethylene, 1,1-diphenyl-1-propene, cyclohexene, and 2-methyl-Δ^1-cyclohexene), on storage in contact with phenyl isocyanate and stannous chloride for periods up to 7 days under perfectly anhydrous conditions gave small quantities of this substituted biuret at room temperature. The *small* yields of biuret are attributable to the predominating polymerization which is favored by the same influences that might accelerate the biuret synthesis. The authors (20) explain the incidence of biuret in this reaction by postulating the primary formation of an unsaturated acid anilide, $CR_2{=}CRCH_2CONHC_6H_5$, which decomposes to provide aniline. Carbanilide, formed therefrom by the excess of phenyl isocyanate, subsequently reacts with more isocyanate, under the powerful catalytic influence of stannic chloride, to give the final product, 1,3,5-triphenylbiuret. The experimental evidence supporting this interpretation is summarized in Section V,G,1(a).

Like the aromatic derivatives, 1,3,5-trialkylbiurets decompose into alkyl isocyanate and 1,3-dialkylurea on pyrolysis (120, 171).

VI. Isobiurets

A. *O*-ethers of biuret

In their pioneer work on isoureas at the turn of last century, Stieglitz and his collaborators, particularly Bruce, Dains, and McKee, first prepared compounds of the isobiuret series. In principle, their general synthesis consists in the condensation of isoureas and isocyanates. By a suitable choice of the starting materials, a wide variety of isobiurets and isothiobiurets are readily available. The parent compound, 2-methylisobiuret, however, was not the first compound of this series to be synthesized. It is obtainable with some difficulty by the interaction of methylisourea hydrochloride and potassium cyanate in aqueous solution; its structure is confirmed by its demethylation, by means of hydrogen chloride, to biuret (62).

$$NH_2C(OCH_3){=}NH + HCNO \rightarrow$$

$$\underset{\text{2-Methylisobiuret}}{NH_2C(OCH_3){=}NCONH_2} \xrightarrow{HCl} \underset{\text{Biuret}}{NH_2CONHCONH_2} + CH_3Cl$$

Madelung and Kern's work (247) on dicyanamide opened a second route to isobiurets. Treatment of sodium dicyanamide, in absolute ethanol, with one equivalent of hydrogen chloride yields, in addition to much polymeric material,

small quantities of the hydrochloride of 1-cyano-2-ethylisourea:

$$NaN(CN)_2 + C_2H_5OH + 2HCl \rightarrow NaCl + CNNHC(OC_2H_5)=NH\cdot HCl$$

Hydrochloride of 1-cyano-2-ethylisourea

The use of an excess of hydrogen chloride affords 2-ethylisobiuret, possibly by loss of ethyl chloride from an intermediate diisobiuret, $NH[C(OC_2H_5)=NH]_2$. No polymeric by-products arise in this reaction, and the free crystalline base is readily isolated from the resulting hydrochloride. Its aqueous solutions are slightly alkaline; addition of hydrochloric acid quickly reprecipitates the hydrochloride (247).

$$NaN(CN)_2 + 2C_2H_5OH + 3HCl \rightarrow NaCl + C_2H_5Cl + NH=C(OC_2H_5)NHCONH_2\cdot HCl$$

More recently, Birkenbach and Kraus (46) obtained crystalline carbamyl isothiocyanate by interacting anhydrous cyanic and thiocyanic acids at 0°C. Successive treatment of this compound with ethanol and ammonia affords *O*-ethylisobiuret in good yields.

$$HN=CO + HNCS \rightarrow \underset{\text{Carbamyl isothiocyanate}}{NH_2CONCS} \xrightarrow{C_2H_5OH} NH_2CONHCS(OC_2H_5) \xrightarrow{NH_3} NH_2CONHC(OC_2H_5)=NH$$

B. DISUBSTITUTED ISOBIURETS

Methyl- and ethylisourea, first obtained by McKee and Stieglitz from cyanamide and alcohol in the presence of an equivalent of hydrogen chloride (245, 372), react rapidly with phenyl isocyanate (in aqueous suspension) to afford fair yields of 4-alkyl-1-phenyl-4-isobiurets. In common with isourea ethers, the products form monohydrochlorides and are dealkylated, with elimination of alkyl chloride, by boiling dilute hydrochloric acid. Small amounts of aniline and ammonia are formed as by-products (61, 245). The method has been used for the preparation of numerous 1-arylbiurets (76, 235) and may be extended to the synthesis of more highly substituted biurets (see below) and thiobiurets.

$$NH_2C(OR')=NH + RNCO \rightarrow RNHCONHC(OR')=NH \xrightarrow{HCl} RNHCONHCONH_2 + R'Cl$$

4-Alkyl-1-phenyl-4-isobiuret (and its 4-thio analog) react with an additional molecule of phenyl isocyanate to form isotriurets and isothiotriurets (*cf.* Section XIII,B,1(e)), respectively (62, 236).

C. TRISUBSTITUTED ISOBIURETS

1-Aryl-2-alkylisoureas are readily synthesized by the passage of hydrogen chloride through a solution of arylcyanamide in a large excess of the appropriate

alcohol. Like other isoureas, they react almost quantitatively with isocyanates to yield 1,2,5-substituted-2-isobiurets, which can be dealkylated to 1,5-disubstituted biurets (245, 371).

$$C_6H_5NHCN + ROH + HCl \rightarrow C_6H_5NHC(OR)=NH \cdot HCl$$

$$C_6H_5NHC(OR)=NH + R'NCO \rightarrow C_6H_5NHC(OR)=NCONHR'$$

$$C_6H_5NHC(OR)=NCONHR' + HCl \rightarrow C_6H_5NHCONHCONHR' + RCl$$

The method has been applied to the preparation of a number of substituted *p*-chlorophenylbiurets, including the biuret analog (XXXIV) of the antimalarial substance Paludrine (76).

XXXIV

D. TETRASUBSTITUTED ISOBIURETS

1,1,2-Trisubstituted isoureas, resulting from the interaction of alkylphenylcyanamides and sodium alkoxide (rather than alcoholic hydrogen chloride), serve, in the same synthesis, as a source of 1,1,5-trisubstituted biurets (245, 372; see also 76).

VII. Thiobiuret and Dithiobiuret

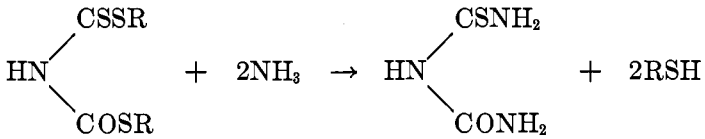
A. THIOBIURET (THIOALLOPHANIC ACID AMIDE, THIOCARBAMIC ACID UREIDE)

Thiobiuret was first synthesized by Wunderlich in 1886 (436) from *N*-cyanourea (173) and ammonium sulfide.

$$NH_2CONHCN + H_2S \rightarrow NH_2CONHCSNH_2$$

The method was described in some detail by Hecht (178; see also 405), but only moderate yields were obtainable in the case of the parent compound itself. The real importance of this synthesis is its wide applicability to the preparation of various substituted thio- and dithiobiurets from the readily accessible intermediate substituted *N*-cyanoureas and *N*-cyanothioureas (*cf.* Sections VIII and X).

Fromm and Junius (142) obtained monothiobiuret as one of the products of the ammonolysis of the dithiobenzyl ester (XXXV).



XXXV Thiobiuret

In an alternative recent synthesis (365) thiobiuret is obtained from guanylurea or its carbonate on treatment with hydrogen sulfide.

$$NH_2CONHC(=NH)NH_2 + H_2S \rightarrow NH_2CONHCSNH_2 + NH_3$$

Guanylurea — Thiobiuret

The conversion is carried out successfully by keeping a solution of the reactant in alcohol at 75°C. under a hydrogen sulfide pressure of 225 lb./sq.in. during 12 hr. (see also Section VII,B,1).

It is of interest to note that an attempt to isomerize urea thiocyanate to thiobiuret was unsuccessful (178).

Thiobiuret is a crystalline solid of intensely bitter taste. It gives the biuret reaction. It is decomposed into cyanourea and hydrogen sulfide by ammoniacal silver nitrate (178, 436).

Thiobiuret is claimed to be a useful rubber accelerator and may serve as an intermediate in the production of thermoplastic and water-repellent resins (365). Condensation products incorporating monothiobiurets have been described (362).

B. DITHIOBIURET

In contrast to the numerous researches concerning the chemistry of substituted dithiobiurets, remarkably little information has been published about the parent compound of this important and interesting group, in spite of the fact that dithiobiuret has been commercially available for some time.

1. Synthesis

In 1892 Hecht (178) described the preparation of a number of substituted dithiobiurets by his now classical cyanourea synthesis, but observed in passing that "unfortunately nothing can be reported about the parent compound, since we have so far not succeeded in preparing the necessary intermediate *N*-cyanothiourea."

Dithiobiuret was first synthesized in the laboratories of the American Cyanamid Company in 1945 (364) by the interaction of dicyandiamide and hydrogen sulfide under pressure in inert solvents. The reaction occurs probably in two stages: it is known that dicyandiamide simply adds the elements of hydrogen sulfide when treated with this gas at atmospheric pressure, to yield guanylthiourea (22, 231, 359), while small quantities of dithiobiuret are formed as a by-product; the yields of dithiobiuret rise slowly as the time of reaction is increased (231).

$$NH_2C(=NH)NHCN + H_2S \rightarrow NH_2C(=NH)NHCSNH_2$$

Dicyandiamide

$$NH_2C(=NH)NHCSNH_2 + H_2S \rightarrow NH_2CSNHCSNH_2 + NH_3$$

Guanylthiourea — Dithiobiuret

Dithiobiuret is also available from the interaction of metallic dicyanamides and hydrogen sulfide. Under various conditions the biuret is obtainable in 44–

64 per cent yields without the use of pressure equipment; it can in fact be produced in a continuous process by running aqueous calcium dicyanamide through packed reaction towers against a stream of hydrogen sulfide. Yields are raised up to 90 per cent by employing closed vessels (237).

Marsh and Hamilton (249) converted calcium dicyanamide into cyanothiourea under carefully controlled conditions, thus succeeding by another route where Hecht (178) had failed sixty years previously. Treatment of 10 per cent aqueous calcium dicyanamide with hydrogen sulfide at 96–97°C. for 1 hr. affords cyanothiourea and dithiobiuret in 55 and 6 per cent yields, respectively. The products are separated by being suspended in an aqueous medium at pH 10; the undissolved dithiobiuret is collected, and the cyanothiourea is recovered by adjusting the hydrogen-ion concentration of the filtrate to pH 2–3.

$$NH(CN)_2 + H_2S \rightarrow NH_2CSNHCN$$

$$NH_2CSNHCN + H_2S \rightarrow NH_2CSNHCSNH_2$$

2. Physical properties (10)

Dithiobiuret forms crystals of apparent density 1.522 ± 0.06 g./cc. at 30°C. It melts with decomposition at 181°C. when heated at a rate of 1–2° per minute from 178°C. Some optical properties of dithiobiuret crystals are summarized in table 5 (10).

Dithiobiuret is practically insoluble in benzene, hexane, and chloroform. Approximate solubility data are shown in table 6. The pH of a saturated aqueous solution of dithiobiuret is 5.8 at 30°C. (10, 237).

TABLE 5

Optical properties of dithiobiuret

Crystal system	Monoclinic or triclinic
Crystal habit	Columnar
Refractive indexes (at 5893 Å.):	
α	1.654 ± 0.003
β	1.880 ± 0.005
γ	> 1.936 (calculated)
Birefringence	Very high; > 0.35
Optical axial angles (at 5893 Å.):	
$2E$	102°
$2V$	52°
Dispersion	Strongly axial

TABLE 6

*Solubility of dithiobiuret**

Solvent	27°C.	50°C.	70°C.	90°C.	100°C.
Water	0.27	0.9	2.5	5.5	8.0
Ethanol	2.2	3.5	4.5		
Acetone	16	17	18		
Cellosolve	34 (ca.)				

* Solubility expressed in grams per 100 g. of solvent.

TABLE 7

Solubility of dithiobiuret in solutions of different pH

Solution	Solubility*
0.4% hydrochloric acid	0.33
Water	0.34
1% sodium hydroxide (0.25 *M*)	3.6 (0.27 *M*)
5% sodium hydroxide (1.25 *M*)	16 (1.19 *M*)
10% sodium hydroxide (2.5 *M*)	29 (2.15 *M*)

* Solubility expressed in grams per 100 g. of solution.

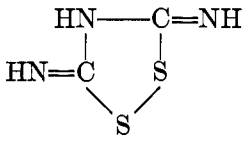
The ultraviolet absorption spectrum of dithiobiuret includes characteristic maxima at 225 mμ (log ϵ = 2.0) and 280 mμ (log ϵ = 2.2), and a minimum at 240 mμ (log ϵ = 1.4); in alkaline solution the absorption bands are displaced somewhat towards the longer wavelengths. The solubility of dithiobiuret in solutions of various pH, estimated from their ultraviolet absorption intensities, is shown in table 7 (10).

The infrared spectrum of dithiobiuret has also been determined in detail (10).

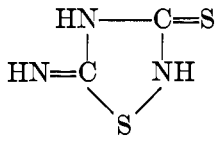
3. Chemical properties

Dithiobiuret is acidic and dissolves in alkalies with formation of water-soluble salts (10).

In common with numerous compounds containing thiol groups capable of forming disulfide linkages on oxidation (2RSH $-$ 2H $\rightleftharpoons$ RSSR), dithiobiuret may act as a strong reducing agent and is reversibly oxidized by such reagents as hydrogen peroxide, iodine, ceric salts, thallic salts, or dithioformamidine salts (307, 308). The resulting oxidation product is regarded as 3,5-diimino-1,2,4-dithiazoline (XXXVI) (308). Its possible alternative formulation as 5-imino-1,2,4-thiadiazolidine-3-thione (XXXVII) (21, p. 47), resulting from the oxidative cyclization involving one thiol group and one imino group, instead of two thiol groups, appears less likely; the oxidation product is instantly decomposed by alkalies with elimination of sulfur, which, in conformity with Fromm's rule (134), is typical of compounds incorporating the $=\overset{|}{C}-S-S-\overset{|}{C}=$ system in their structure.



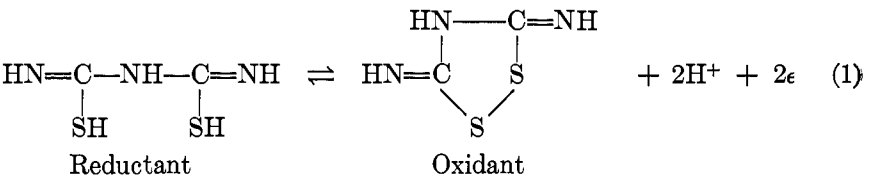
XXXVI



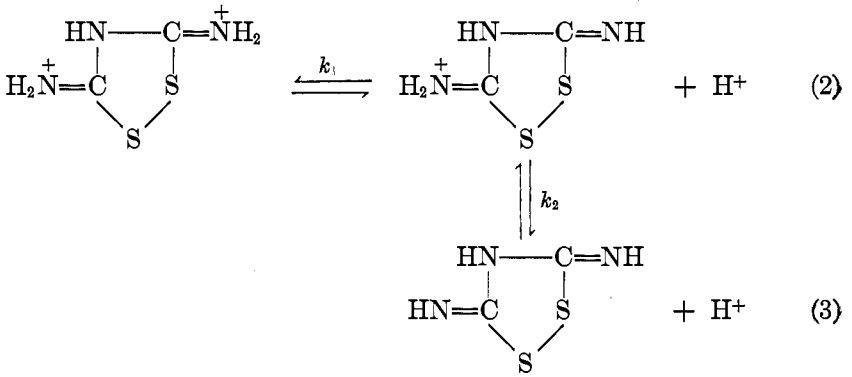
XXXVII

Following their detailed work on the reversible oxidation of thiourea to dithioformamidine (309) Preisler and Bateman (308) thoroughly investigated the

reversible oxidation of dithiobiuret (equation 1) and measured the oxidation–reduction potentials of this system in the pH region 0.05 to 5.2.



While dithiobiuret is not appreciably ionized over this pH range, the oxidation product behaves as a dibasic acid, dissociating in the following two stages:



For the purpose of discussion, the primary ionization (equation 2) was assumed to be that of a relatively strong acid, while the secondary ionization (equation 3) corresponded to that of a weak acid ($k_2 = 4 \times 10^{-8}$; $pK_2 = 7.4$).

The oxidation of dithiobiuret was found to proceed by a reversible two-step process, apparently without intermediate semiquinone formation. The observed oxidation–reduction potentials of the thiol–disulfide system agreed satisfactorily (over the pH range 0.05 to 5.2) with the theoretically derived equation (equation 4) applying to systems involving a reversible two-equivalent change from reductant to oxidant:

$$E_h = E_0 + 0.03 \log \frac{C_0}{C_r} + 0.03 \log \frac{[H^+]^2}{[H^+]^2 + k_1[H^+] + k_1 k_2} \tag{4}$$

$$C_0 = [Ox] + [OxH^+] + [OxH_2^{++}] \tag{5}$$

The symbols have the following significance:

E_h = observed potential (referred to the normal hydrogen electrode as zero),
C_r = molecular concentration of the reductant,
C_0 = molecular concentration of the oxidant (consisting of the sum of the concentrations of the undissociated molecules, monovalent ions, and divalent ions, as expressed by equation 5),
E_0 = a constant, characteristic of the system, depending on the value of the dissociation constants k_1 and k_2.

From the measurements, the E_0' value[4] of the system, at pH 0, was estimated to be +0.251 v. The corresponding oxidation–reduction potential of the thiourea–dithioformamidine system is $E_0' = 0.420$ v. (309). As predicted by the potentials of these two systems, dithioformamidine salts readily oxidize dithiobiuret, the expected potentials being rapidly and accurately attained; thiol (—SH) and dithio (—S—S—) systems are thus capable of reacting rapidly and reversibly with one another.

The results of Preisler and Bateman show that dithiobiuret is one of the strongest organic reducing agents in the pH range up to 5.2. In solutions of lower hydrogen-ion concentration, however, the heterocyclic oxidant is decomposed with liberation of sulfur; this limits the usefulness of dithiobiuret as a reducing agent in alkaline media.

Of the numerous reactions which dithiobiuret may be expected to undergo, only relatively few have been studied.

Alkylation of dithiobiuret by means of dimethyl sulfate or benzyl chloride yields the appropriate *S*-alkylated isodithiobiurets (10), which are also obtainable by the interaction of isothioureas and isothiocyanates (407; see also Section XI,B).

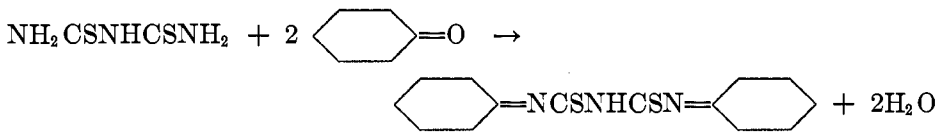
$$\underset{\text{Dithiobiuret}}{NH_2CSNHCSNH_2} + RCl \rightarrow NH_2CSNHC(=NH)SR + HCl$$

Dithiobiuret condenses readily with formaldehyde to yield a product which is regarded as 1,5-bis(hydroxymethyl)dithiobiuret (366), although a structure incorporating *S*-methylol groupings is not definitely excluded (303).

$$NH_2CSNHCSNH_2 + 2HCHO \rightarrow \underset{\text{1,5-Bis(hydroxymethyl)dithiobiuret}}{HOCH_2NHCSNHCSNHCH_2OH}$$

With aromatic aldehydes, dithiobiuret yields condensation products having triazine structures. A series of six compounds, thus prepared, showed antitubercular activity *in vitro* (127; concerning structure, *cf.* 114) (see also Section X,A,2).

The reaction of dithiobiuret with ketones has been claimed to give rise to dialkylidene derivatives: cyclohexanone, for example, affords dicyclohexylidenedithiobiuret (10; but compare 114).



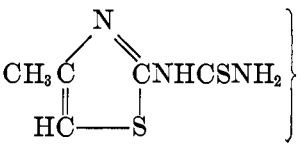
Dicyclohexylidenedithiobiuret

The classical aminothiazole synthesis, from thiourea and chloroacetaldehyde (396), has been extended to dithiobiuret, which reacts with chloroacetone with

[4] E_0' is customarily defined as the E_h of an equimolecular mixture of oxidant and reductant at some specified pH.

elimination of water and hydrogen chloride to form 2-thioureido-4-methylthiazole hydrochloride in 96 per cent yield (368).

$$\underset{\text{Dithiobiuret}}{NH_2CSNHCSNH_2} + \underset{\text{Chloroacetone}}{CH_2ClCOCH_3} \rightarrow$$



$$\} \; HCl + H_2O$$

4-Methyl-2-thioureido-thiazole hydrochloride

4. Uses

Dithiobiuret is a useful insecticide, plasticizer, rubber accelerator, and intermediate for the production of thermoplastic and water-repellent resins (59, 364). Owing to its reducing properties it may be of potential value as a metal deactivator (10) and is used, for the same reason, to minimize color formation in solutions of polyacrylonitrile in dimethylformamide (417). Other uses are suggested by its physiological and pharmacological properties (*cf.* Section XV,B).

VIII. Substituted Thiobiurets

Owing to the unsymmetrical structure of monothiobiuret, due to the presence of both oxygen and sulfur atoms in the molecule, the number of theoretically possible position isomers derived from it is large. However, the chemistry of substituted thiobiurets has received comparatively little attention, and members of but a few of the seventeen possible series of thiobiuret derivatives (substituents being identical) have been investigated. They are usually prepared by variations of the general syntheses; their properties conform to the general pattern of biuret chemistry.

A. 1-Substituted-2-thiobiurets

The general method of demethylating *O*-alkylisobiurets has been employed in the preparation of 1-substituted-2-thiobiurets (*cf.* Section IX).

Recent work on carbamyl isothiocyanate has provided an alternative route. In its reaction with aniline at −10°C. in ethereal solution, this isothiocyanate affords 1-phenyl-2-thiobiuret almost quantitatively (46).

$$\underset{}{C_6H_5NH_2} + \underset{\text{Carbamyl isothiocyanate}}{SCNCONH_2} \rightarrow \underset{\text{1-Phenyl-2-thiobiuret}}{C_6H_5NHCSNHCONH_2}$$

B. 1-Substituted-4-thiobiurets

Soon after its discovery, the cyanourea synthesis (178, 436) was successfully applied (178) to the preparation of 1-methyl- and 1-ethyl-4-thiobiurets. In this general procedure, 1-substituted-3-cyanoureas are converted, on treatment with ammonia and hydrogen sulfide in the presence of ammonium chloride, to the

appropriate thiobiurets, though often in unsatisfactory yields (compare 52, 358).

$$RNCO + NH_2CN \rightarrow RNHCONHCN \xrightarrow{H_2S} RNHCONHCSNH_2$$

The condensation of phenyl isocyanate and thiourea at 100°C. affords good yields of 1-phenyl-4-thiobiuret. The method fails with mono- and disubstituted thioureas, carbanilide and isothiocyanates being formed as main products (236). Attempts to produce dithiobiurets by the corresponding reaction of isothiocyanates have also been unsuccessful (77).

$$RNCO + NH_2CSNH_2 \rightarrow RNHCONHCSNH_2$$

1-Aryl-4-thiobiurets are soluble in strong alkalies and reprecipitated therefrom by carbon dioxide (52). The lower alkyl homologs dissolve in both acids and bases and, probably because of their solubility in water (178), are not reprecipitated on dilution. The majority of these derivatives possess an intensely bitter taste (52, 178).

The thiol group of thiobiurets is readily alkylated on treatment with alkyl halides in closed vessels (236). The exchange of a thiol by a hydroxy group is a general reaction which converts thio- and dithiobiurets into the corresponding biuret derivatives. Alkaline lead salts (99), alcoholic silver nitrate (99), lead hydroxide (236), or ammoniacal silver nitrate (99, 178) are all effective desulfurizing agents.

c. 1,1-DISUBSTITUTED-4-THIOBIURETS

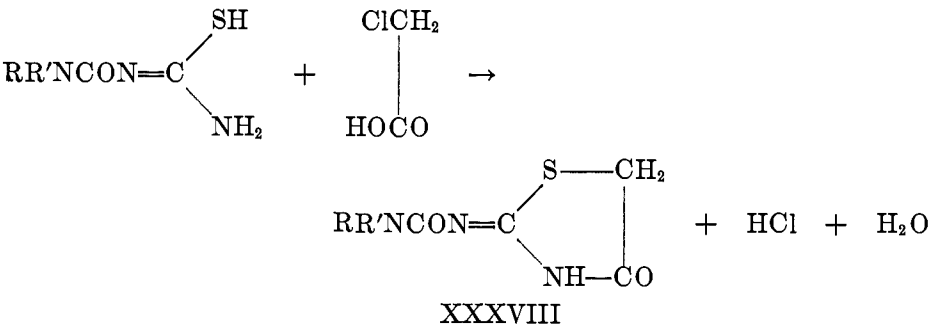
Diphenylcarbamyl chloride and its homologs react with mercuric thiocyanate in high-boiling hydrocarbon solvents, such as cumene, to yield solutions of the corresponding isothiocyanates (99) formed by isomerization of intermediate thiocyanates (*cf.* 213). Efforts to isolate these products in the pure state were unsuccessful; they react with ethanolic ammonia *in situ*, however, to yield thiobiurets. The use of aliphatic or aromatic primary amines afforded a variety of 1,1,5-trisubstituted-4-thiobiurets, but, except in one instance, the method failed with secondary amines. In actual practice the preparation of the carbamyl isothiocyanate solutions is a matter of some difficulty; some mercuric sulfide is invariably carried over and must be removed from the final product. The yields are variable but exceed 60 per cent of the theoretical in some cases (99; compare also 213).

$$RR'NCOCl \xrightarrow{Hg(SCN)_2} [RR'NCOSCN] \rightarrow RR'NCONCS \xrightarrow{NH_3} RR'NCONHCSNH_2$$

Attempts to vary this synthesis, by condensing diphenylcarbamyl chloride and thioureas directly under a variety of conditions, did not give the desired results (99).

1,1-Disubstituted-4-thiobiurets are soluble in alkalies and reprecipitated by acids (99). Like other structures incorporating the thioureido grouping, they

condense with α-substituted ketonic compounds (such as chloroacetic acid) to yield thiazole derivatives of the type of XXXVIII. Although reaction does not occur with ethyl chloroacetate in ethanol, it proceeds readily when a slight excess of the free acid is allowed to act upon the thiobiuret until no more hydrogen chloride is evolved (99).



XXXVIII

D. 1,5-DISUBSTITUTED-2-THIOBIURETS (*cf.* SECTION IX,B)

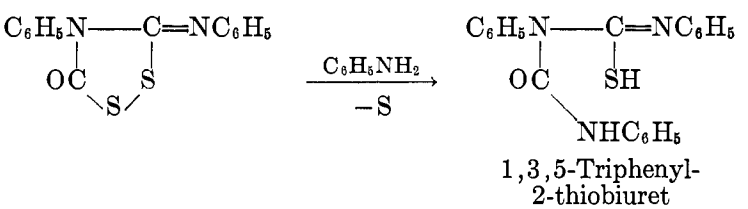
E. 1,1,5-TRISUBSTITUTED-4-THIOBIURETS

1,1,5-Trisubstituted-4-thiobiurets, obtainable by the interaction of carbamyl isothiocyanates and amines (*cf.* Section VIII,C), are crystalline solids; they are soluble in alkalies and are reprecipitated by acids (99). Desulfurization by ammoniacal silver nitrate affords the corresponding biurets. In the presence of a large excess of ammonia, however, replacement of the eliminated thiol group by an amino group results in the formation of the appropriate guanylureas (99).

$$RR'NCONHCSNHR'' + 2AgNO_3 + NH_3 \rightarrow RR'NCONHC(=NH)NHR'' + Ag_2S + 2HNO_3$$

F. 1,3,5-TRISUBSTITUTED-2-THIOBIURETS

4-Alkyl-5-oxo-3-alkylimino-1,2,4-dithiazolidines (and their aromatic analogs) are readily prepared by the action of bromine on isothiocyanates in chloroform containing small proportions of ethanol (131, 132; concerning structures compare 175). In boiling aniline their heterocyclic nucleus is opened, with elimination of hydrogen sulfide and formation of substituted thiobiurets; alkyl groups are simultaneously replaced by phenyl residues. 1,3,5-Triphenyl-2-thiobiuret has thus been obtained from the appropriate methyl-, ethyl-, and phenyl-substituted 1,2,4-dithiazolidines (131, 132, 175).



1,3,5-Triphenyl-2-thiobiuret

IX. Isothiobiurets

A. Disubstituted isothiobiurets

McKee's general method (245) of synthesizing isobiurets is readily extended to isothiobiurets. Whereas methylisourea is capable of condensing with two molecules of phenyl isocyanate, it reacts with one molecule of isothiocyanate only (62). Demethylation of the resulting isothiobiuret, by hydrogen chloride at 75–90°C., affords 1-substituted-2-thiobiurets (62). More highly substituted isoureas react with equal facility (77).

$$NH_2C(OCH_3)=NH + RNCS \rightarrow RNHCSNHC(OCH_3)=NH \xrightarrow{HCl} RNHCSNHCONH_2 + CH_3Cl$$

Methylisourea

The preparation of 2-methyl-1-phenyl-2-isothiobiuret by the acid hydrolysis of 3-cyano-2-methyl-1-phenylisothiourea has been claimed (414); thiohydrolysis does apparently not occur under these conditions.

$$C_6H_5N=C(SCH_3)NHCN + H_2O \rightarrow C_6H_5N=C(SCH_3)NHCONH_2$$

3-Cyano-2-methyl-1-phenylisothiourea — 2-Methyl-1-phenyl-2-isothiobiuret

1,4-Disubstituted-4-isothiobiurets have been prepared by the alkylation of 1-phenyl-4-thiobiuret, using methyl iodide under pressure (236).

B. Trisubstituted isothiobiurets

1,2,5-Trisubstituted isothiobiurets are obtainable by McKee's method (245) in the usual manner, and are thiohydrolyzed by the general procedures (*cf.* Section XI) to disubstituted thiobiurets (236, 269; see also 77, 152). Simultaneous desulfurization and ammonolysis (effected by ammonia, in the presence of mercuric oxide) yield substituted guanylureas (XXXIX) (77).

$$C_6H_5NHCSNHCONHC_6H_5 + CH_3SH$$

$$\uparrow KHS$$

$$C_6H_5NHC(SCH_3)=NH + C_6H_5NCO \rightarrow C_6H_5NHC(SCH_3)=NCONHC_6H_5$$

$$\downarrow NH_3, HgO$$

$$C_6H_5NHC(=NH)NHCONHC_6H_5$$

XXXIX

X. Substituted Dithiobiurets

A. 1-Substituted dithiobiurets

1. Synthesis

(a) The isoperthiocyanic acid synthesis

In an investigation concerning "isoperthiocyanic acid" (432) [3-imino-5-thio-1,2,4-dithiazolidine] Glutz observed (163) that its interaction with aniline

above 100°C. gave a phenylated dithiobiuret, while sulfur was liberated. The occurrence of thiocarbanilide as a by-product was favored by the presence of an excess of arylamine (133).

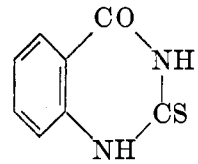
$$C_2H_2N_2S_3 + C_6H_5NH_2 \rightarrow \underset{\text{1-Phenyldithiobiuret}}{C_6H_5NHCSNHCSNH_2} + S$$

$$C_2H_2N_2S_3 + 4C_6H_5NH_2 \rightarrow \underset{\text{Thiocarbanilide}}{2(C_6H_5NH)_2CS} + 2NH_3 + S$$

Analogous results were observed by Tursini in experiments with *p*-toluidine (404), but the representation of these compounds as 1-aryldithiobiurets was not confirmed until Hecht provided dithiobiurets by an unequivocal synthesis (*cf.* Section X,A,1(b)) for comparison (178). The reaction was subsequently developed by Fromm and his school, who evolved optimum experimental conditions for the synthesis of numerous 1-aryldithiobiurets (133, 139, 140, 146, 147, 149) and reported the results of extensive researches into this class of compounds over a period of thirty years.

The synthesis proceeds normally with secondary amines (*cf.* Section X,B,1), but a different reaction occurs when tertiary amines are employed: isoperthiocyanic acid and dimethylaniline, for example, react to yield 4,4′-bis(dimethylamino)diphenyl sulfide, $[—C_6H_4N(CH_3)_2]_2S$ (404). The method fails in the case of aliphatic amines (e.g., methylamine and allylamine) (378) and cannot, of course, be modified to provide 1-aryl-5-alkylbiurets (77).

In later years the reaction has been widely used (47, 116, 168, 377, 378). Particularly extensive papers on this subject are due to Underwood and Dains (406) and Fairfull and Peak (113). Experience has shown that certain amines may yield 1,3-disubstituted thioureas or heterocyclic products as main products in this reaction. Thioureas result from *p*-xenylamine and benzylamine, for example, while anthranilic acid and *o*-phenylenediamine yield 4-keto-2-thiotetrahydroquinazoline (XL) and *o*-phenylenethiourea, respectively (406). Numerous arylamines, particularly those having very feebly basic properties, fail to react at all (113, 406).



XL

Crude products prepared by the isoperthiocyanic acid synthesis are frequently contaminated by sulfur, which cannot easily be removed by crystallization. Precipitation of the dithiobiurets from dilute sodium hydroxide with hydrogen sulfide effects rapid purification, often with improved yields (113).

(b) The cyanourea synthesis

Hecht and Wunderlich's cyanourea synthesis is readily applicable to the preparation of 1-substituted dithiobiurets, and was in fact first applied to this purpose by its discoverers (178, 436). The condensation of isothiocyanates and sodium cyanamide affords the required intermediate 1-substituted-3-cyanothioureas. Their subsequent treatment, in ammoniacal solutions containing ammonium chloride, with hydrogen sulfide produces dithiobiurets in fair yields (178, 436; compare also 150).

$$RNCS + NHNaCN \rightarrow RN{=}C(SNa)NHCN \xrightarrow{H_2S} RNHCSNNaCSNH_2$$

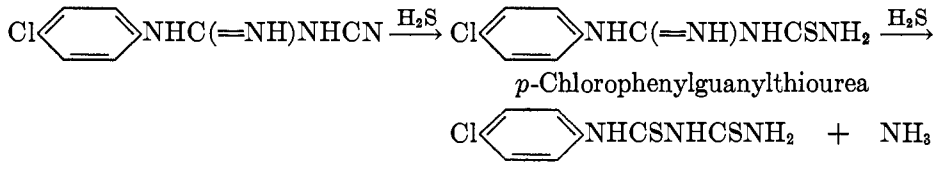
The reaction has been widely employed for the preparation of numerous alkyl, aryl, and heterocyclic derivatives (113, 378). Structures incorporating quaternary ammonium groups are accessible by the preliminary quaternization of an isothiocyanato-substituted base, followed by successive treatment with sodium cyanamide and hydrogen sulfide (113).

A valuable modification of this synthesis, which by-passes the isolation of isothiocyanates, has been developed by Fairfull and Peak (113), who prepared *N*-cyanothioureas by an alternative route. Dithiocarbamates of the type RNHCSSR′ (which are known to decompose to the isothiocyanate, RNCS, and the thiol, R′SH, on warming) were directly acted upon with sodium cyanamide in boiling methanol or ethanol. The elements of hydrogen sulfide were then added to the resulting *N*-cyanothioureas in the usual way.

A convenient method of converting cyanides into thioamides, recently described by the same authors (112), is applicable to the synthesis of dithiobiurets. This reaction occurs with remarkable ease, and is usually complete within 2-4 hr. at room temperature, when hydrogen sulfide is passed through a solution of the cyanide in pyridine containing a strong base such as triethylamine. The treatment of 1-*p*-chlorophenyl-3-cyano-2-ethylisothiourea results in the addition of hydrogen sulfide and simultaneous thiohydrolysis (*cf.* Section XI), with production of 1-(*p*-chlorophenyl)dithiobiuret.

(c) From aryldicyandiamides

Small quantities of 1-substituted dithiobiuret have been isolated as a by-product in the production of *p*-chlorophenylguanylthiourea from the dicyandiamide (47). The reaction, which appears to be favored by the presence of sodium ethoxide, is comparable with the formation of dithiobiuret in the corresponding reaction of the parent compounds (231).



p-Chlorophenylguanylthiourea

1-(*p*-Chlorophenyl)dithiobiuret

(d) From 1,4-disubstituted-4-isodithiobiurets (*cf.* Section XI,B)

2. Properties

1-Substituted dithiobiurets display predominantly acidic but also feebly basic properties. They are soluble in alkalies and ammonia and are reprecipitated by acids. The lower 1-alkyldithiobiurets are soluble in water and may be recrystallized therefrom (133, 163, 436). The supposed hydrochloride of 1-phenyldithiobiuret, prepared by dissolution of the free base in aqueous ferric chloride, is undoubtedly an oxidation product. Oxalates, thiocyanates, and nitrates have been mentioned (163, 404) in the literature. 1-Dithiobiurets are generally neutral towards indicators and often possess an intensely bitter taste (178). Their melting points vary with the rate of heating and must be taken under standard conditions to ensure reproducible results (113).

Acylation of 1-aryldithiobiurets by acetyl chloride (404) or acetic anhydride (145) yields monoacetyl and diacetyl derivatives of unknown constitution.

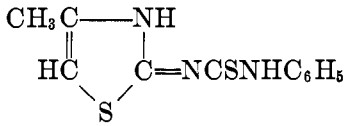
One or both of the sulfur atoms of 1-substituted dithiobiuret, reacting in the tautomeric thiol form, may be alkylated (146, 404) (see Section XI,A, C, and F). Because of its strong desulfurizing action, benzyl chloride reacts anomalously: in alkaline (135, 146, 149) or ammoniacal (149) solution, dithiobiurets lose the elements of hydrogen sulfide, with the formation of 1-aryl-2-benzyl-3-cyanoisothiourea. 1-Methyl-1-phenyldithiobiuret reacts analogously, except that the 2-thiol group is not benzylated (135).

$$ArNHC(SH)=NC(SH)=NH + C_7H_7Cl + 3NaOH \rightarrow$$
$$ArNHC(SC_7H_7)=NCN + NaCl + Na_2S + 3H_2O$$
$$Na_2S + 2C_7H_7Cl \rightarrow 2NaCl + (C_7H_7)_2S$$

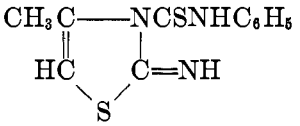
Ethylene chlorohydrin in alkaline solution acts as an even stronger desulfurizing agent and converts 1-phenyldithiobiuret into 3-cyano-1-phenylurea (together with varying quantities of 1,3-diphenylurea and triphenylisomelamine) (150). This desulfurization is, of course, also effected by sodium plumbite in strongly alkaline solution (139). Finally, the use of suitable desulfurizing agents, including freshly precipitated silver oxide, mercuric oxide, or lead oxide, in the presence of ammonia affords dicyandiamides. The reaction occurs particularly rapidly when the reagent (such as silver oxide) remains dissolved in the reaction mixture (377).

$$RNHCSNHCSNH_2 + NH_3 + 2Ag_2O \rightarrow$$
$$RNHC(=NH)NHCN + 2Ag_2S + 2H_2O$$

With chloroacetone 1-aryldithiobiurets yield thiazole derivatives; on the basis of the experimental evidence, Fromm and Phillipe (145) eliminated several possible formulas for those products, but were not able finally to decide between structures XLI and XLII.



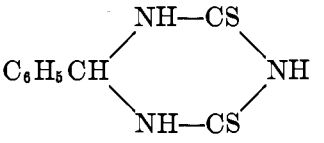
XLI



XLII

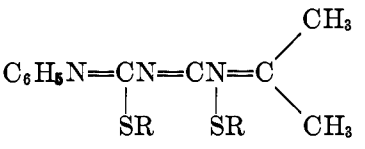
Condensation with carbonyl compounds ("aldurets" and "keturets")

1-Aryldithiobiurets condense readily with aldehydes and ketones, with elimination of water, under the influence of hydrogen chloride (133) or boron trifluoride plus acetic acid (114). The reaction was first investigated by Fromm (133), who suggested the generic names "aldurets" and "keturets" for the resulting products. A compound identical with the condensation product of dithiobiuret and benzaldehyde had been prepared earlier by Brodski (60; compare also 114) by the fusion of a mixture of benzaldehyde and ammonium thiocyanate, and was formulated by this investigator as a triazine (XLIII).

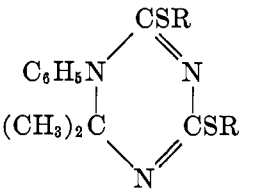


XLIII

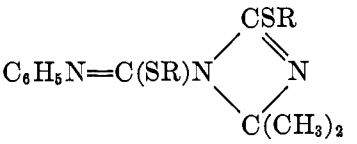
Fromm first established that his condensation reaction involved the nitrogen and not the sulfur atoms of the dithiobiurets: the product from phenyldithiobiuret and acetone, for example, was shown to contain two free thiol groups, since its dibenzyl derivative yielded the dithiobenzyl ester $NH(COSC_7H_7)_2$, and thence benzylthiol, on hydrolysis (133). On this basis Fromm considered three possible structures (XLIV, XLV, and XLVI: R=H), and, unlike Claus (72), favored XLVI as the most satisfactory representation of all the properties of the "keturet."



XLIV



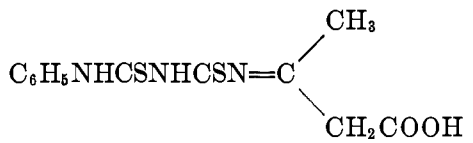
XLV



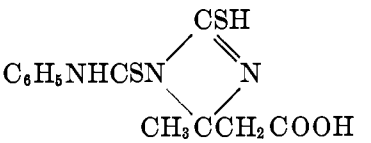
XLVI

The existence of aldurets and keturets derived from 1-methyl-1-phenyldithiobiuret (142) was held to be particularly strong evidence against the triazine structure (XLV), since both substituents cannot be accommodated on the same nitrogen atom in the heterocyclic nucleus. Evidence against structure XLIV seemed provided by the observation (145) that the keturet from 1-phenyldithiobiuret and ethyl acetoacetate could be hydrolyzed to the corresponding

acid, which had no tendency for cyclodehydration. This was regarded as improbable for the structure XLIVa but not unlikely for the structure XLVIa.



XLIVa



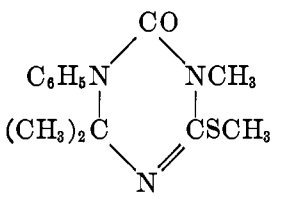
XLVIa

The formation of numerous aldurets and keturets, derived from 1-*o*-tolyl- (140), 1-*p*-tolyl-2,4-dimethyl-2,4-diiso- (146), 1-*m*-tolyl-, and 1-α-naphthyldithiobiuret (407), was similarly interpreted by Fromm and his school.

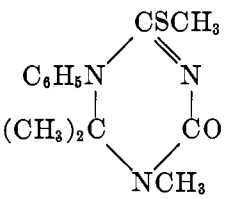
However, Fairfull and Peak (114) have recently adduced evidence in favor of a hexahydro-4,6-thiono-1,3,5-triazine structure (XLV: R=H) for Fromm's "aldurets" and "keturets." The keturet from 1-phenyldithiobiuret and acetone was converted into the di-*S*-methyl derivative (XLIV, XLV, or XLVI: R = CH_3). This afforded a monomethiodide, which was in turn degraded to an *N*-methylamide as follows:

$$-C(SCH_3)=N- \xrightarrow{CH_3I} -C(SCH_3)=\overset{+}{N}CH_3-\}I^{\ominus} \xrightarrow{NaOH} -CONCH_3- + NaI + CH_3SH$$

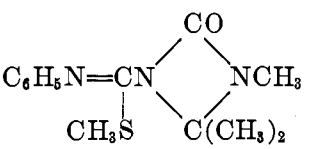
Acid hydrolysis of the *N*-methylamide gave aniline and *not* *N*-methylaniline; this proved that the *N*-phenyl group was not involved and allowed the following four possible structures for the *N*-methylamide (XLVII to L).



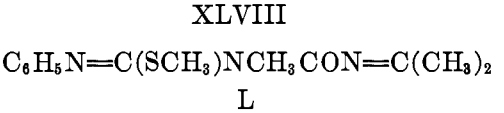
XLVII



XLVIII



XLIX

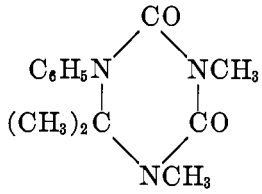


L

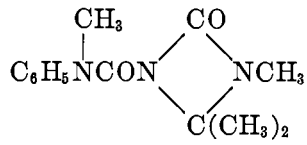
The observed formation of a second hydrolysis product, 1-methyl-3-methylthiocarbonylurea ($CH_3NHCONHCOSCH_3$), eliminated structures XLVII and L, thereby also eliminating XLIV as a possible structure of the parent keturet.

A choice between structures XLVIII and XLIX was made as follows: Quaternization of the *N*-methylamide (XLVIII or XLIX), followed by treatment with alkali, introduced, as above, a second methylamide grouping, yielding the diamide (LI or LII). The low methoxyl content (Zeisel determination) of this diamide incidentally confirmed it to be an *N*-methyl and *not* an *O*-methyl derivative. Acid hydrolysis of this diamide (LI or LII) gave aniline and *not*

N-methylaniline. Structures LII and thence XLIX and XLVI are therefore eliminated.



LI



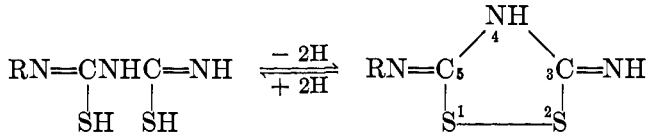
LII

The arguments thus clearly lead to the hexahydrotriazine structure of the keturet from 1-phenyldithiobiuret and acetone. It appears reasonable to assume that other such compounds have this ring structure whenever this is possible. Condensation products from 1,1-disubstituted dithiobiurets, however, cannot be included in this class, and further experimental evidence concerning these derivatives is required before their structure can be definitely established.

Foye and Hefferen (127) have recently prepared a number of analogous condensation products of dithiobiurets and aldehydes by heating the reactants in acetic acid. Their assumed structure (XLIV), presented without evidence, must be rejected, since their product from 1-phenyldithiobiuret and benzaldehyde, for example, is identical with Fairfull and Peak's (114) compound.

Oxidation

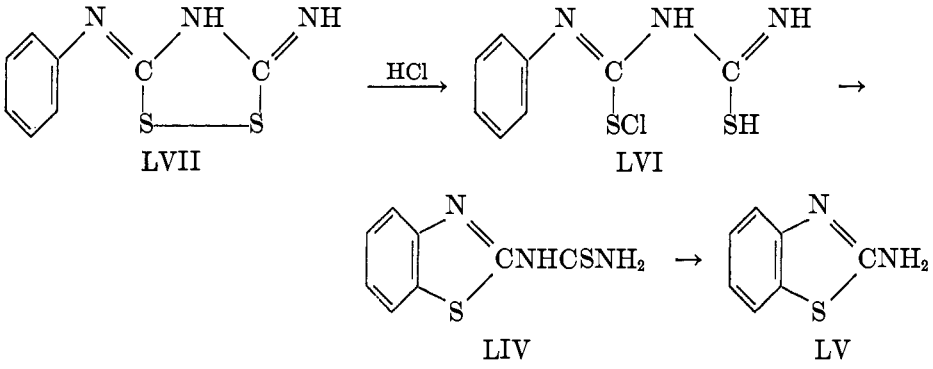
It was first shown by Fromm (133) that 1-aryldithiobiurets react with two equivalents of oxidizing agents such as iodine, ferric chloride, potassium ferricyanide, or hydrogen peroxide in neutral or acid solution to yield products for which the generic name "thiurets" was adopted. Of the possible formulas Fromm selected the 1,2,4-dithiazolidine structure (LIII) as agreeing most satisfactorily with the chemical behavior of these oxidation products (133).



LIII

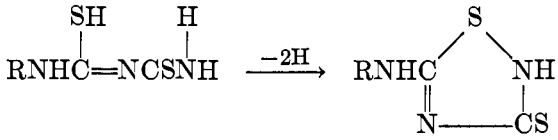
"Phenylthiuret" (LIII: R = C_6H_5), for example, was a labile base which yielded aniline and ammonia on alkaline hydrolysis, and was almost quantitatively reconverted to 1-phenyldithiobiuret on reduction with nascent hydrogen. Concentrated hydrochloric acid at 165°C. gave hydrogen sulfide, carbon dioxide, ammonia, and 2-aminobenzthiazole (LV). Although the latter observation gives some support to the alternative 2-thioureidobenzthiazole (LIV) structure for "phenylthiuret," this formulation appears to be excluded by the results of the alkaline hydrolysis; it is unlikely that aniline should be obtainable from LIV, since the structural analog, 2-aminobenzthiazole (LV), yields *o*-aminothiocresol and *not* aniline under identical conditions. Fromm (133) accordingly interpreted

the hydrochloric acid hydrolysis of "phenylthiuret" by the sequence LVII → LVI → LIV → LV (see also 146).

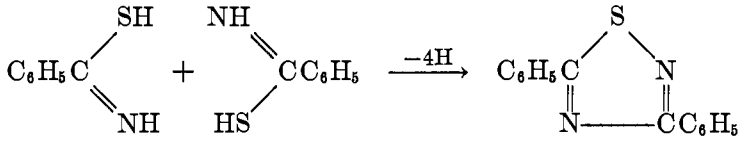


LVII LVI LIV LV

Fromm and his school (139, 140, 146, 147, 148, 149) and later investigators (378, 407) described the oxidation of numerous dithiobiurets and investigated the properties of the resulting products in considerable detail. The latter possess the great reactivity expected of compounds incorporating disulfide linkages flanked by double bonds (134). Their heterocyclic nuclei are opened by alkalies, amines, phenylhydrazine (140, 147, 148), and hydrazine hydrate (144) with elimination of sulfur and formation of a variety of substituted guanylthioureas and triazoles. Attention has recently been drawn by Bambas (21, pp. 44–51) to the possible alternative ring-closure of aryldithiobiurets on oxidation to yield 1,2,4-thiadiazoles.



He cited Hofmann and Gabriel's (194) and Walther's (413) oxidation of thiobenzamide to 3,5-diphenyl-1,2,4-thiadiazole in support of this interpretation and discussed the advantages of this formulation in connection with the struc-



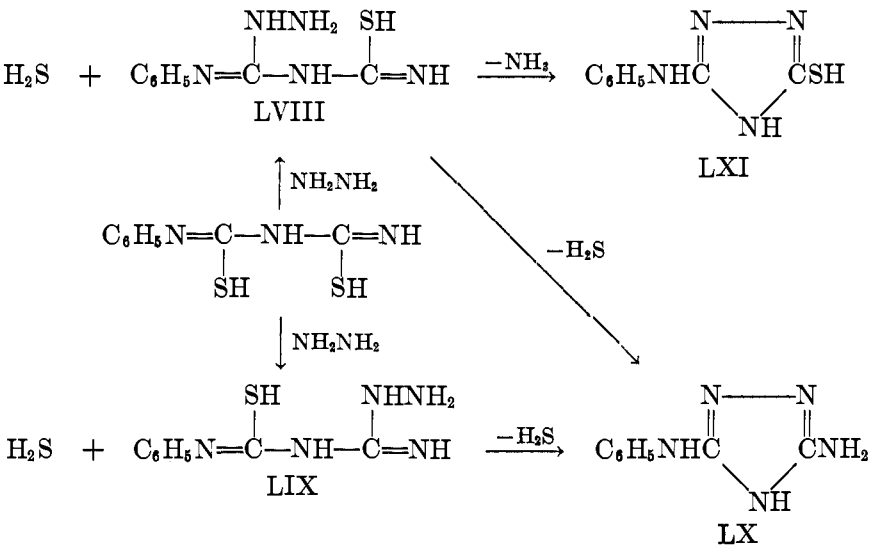
3,5-Diphenyl-1,2,4-thiadiazole

tures of perthiocyanic and isoperthiocyanic acids. In view of the ease with which "thiurets" undergo ring fission, on the one hand, and the relative stability of substituted 3,5-diamino-1,2,4-thiadiazoles, on the other (232), Fromm's cyclic disulfide structure appears to be preferable until more direct experimental evidence becomes available (see also Section VII,B,3).

Concentrated hydrazine hydrate splits 1-aryl- and 1-aryl-1-alkyldithiobiurets according to the following equation (139):

$$RR'NCSNHCSNH_2 + NH_2NH_2 \rightarrow RR'NCSNH_2 + NH_2NHCSNH_2$$

Dilute hydrazine, in aqueous or alcoholic solution, reacts with phenyldithiobiuret (or its oxidation product, "phenylthiuret"), with evolution of ammonia and hydrogen sulfide and with formation of two isomeric substituted guanylthioureas (LVIII and LIX) as primary products. Either of these compounds undergoes ring-closure, with loss of hydrogen sulfide, to yield 3-amino-5-phenylamino-1,2,4-triazole (LX). The former isomer also eliminates the elements of ammonia to form 5-phenylamino-3-thiol-1,2,4-triazole (LXI), which is, incidentally, the main product of this reaction (15, 137, 139, 144).



B. 1,1-DISUBSTITUTED DITHIOBIURETS

1. Synthesis

The isoperthiocyanic acid synthesis (*cf.* Section X,A,1(a)) is readily applicable to the preparation of 1,1-disubstituted dithiobiurets. At 110°C. methylaniline or ethylaniline and isoperthiocyanic acid, for example, afford 1-alkyl-1-phenyldithiobiurets almost quantitatively (138, 141; see also 378).

2. Properties

Like 1-phenyldithiobiuret, the 1-ethyl-1-phenyl homolog is completely desulfurized by ethylene chlorohydrin in alkaline solution (150).

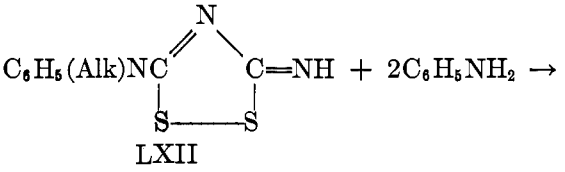
$$C_6H_5NCSNHCSNH_2 \rightarrow C_6H_5NCSNHCN \rightarrow C_6H_5NCONHCN$$
$$\quad\quad C_2H_5 \quad\quad\quad\quad C_2H_5 \quad\quad\quad\quad C_2H_5$$

On treatment with benzylthiol and hydrogen chloride, 1-methyl-1-phenyldithiobiuret is split into *N*-methylaniline and thiobenzyl 1,3-dithioallophanate.

Fromm's attempts to convert this ester into dithiobiuret by ammonolysis failed, however, since the compound was immediately decomposed by ammonia into benzylthiol and thiocyanic acid (136).

$$C_6H_5N(CH_3)CSNHCSNH_2 + HCl + C_7H_7SH \rightarrow$$
$$C_6H_5NHCH_3 \cdot HCl + C_7H_7SCSNHCSNH_2$$
$$C_7H_7SCSNHCSNH_2 \rightarrow C_7H_7SH + 2HCSN$$

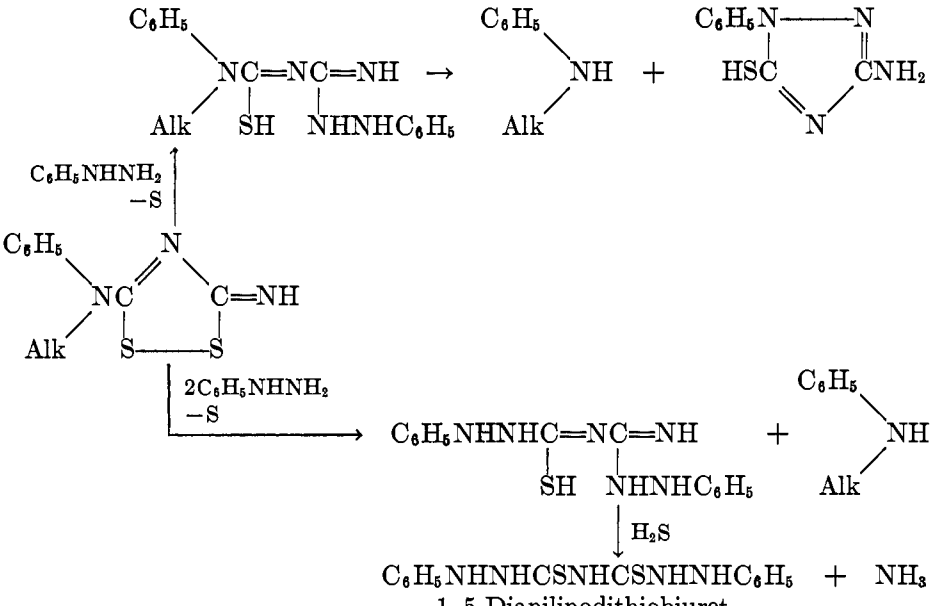
The oxidation of dithiobiurets to 1,2,4-dithiazolidines occurs with the usual facility in the case of 1,1-disubstituted dithiobiurets (138, 141). The heterocyclic nuclei of these oxidation products are cleaved by a number of reagents, and in certain cases dithiobiuret derivatives are regenerated. Aniline splits 3-imino-5-phenylalkylamino-1,2,4-dithiazolidine (LXII) with elimination of sulfur; the primary amine enters the molecule at the carbon atom vacated by the sulfur, and simultaneously displaces the 5-phenylalkylamino group. 3-Phenyl-1-phenylguanylthiourea is therefore formed by both phenylmethyl- and phenylethyl-"thiuret" (LXII) (138).



LXII

$$C_6H_5(Alk)NH + S + C_6H_5NHCSNHC(=NH)NHC_6H_5$$

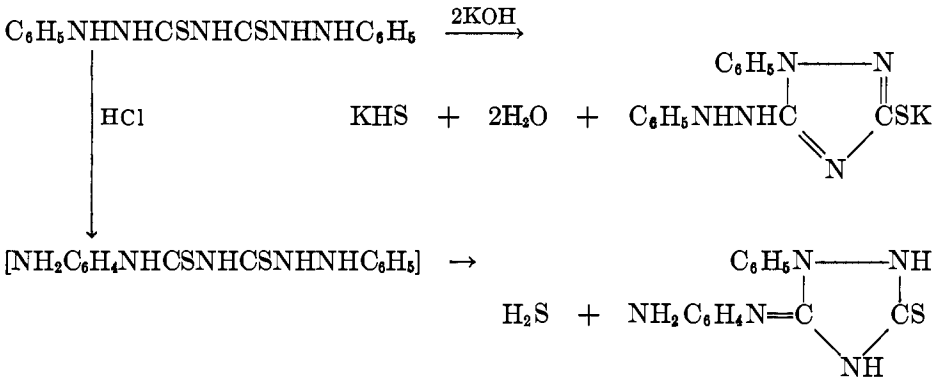
In the cleavage brought about by phenylhydrazine, two reactions take place simultaneously, involving one or two molecules of the hydrazine, respectively



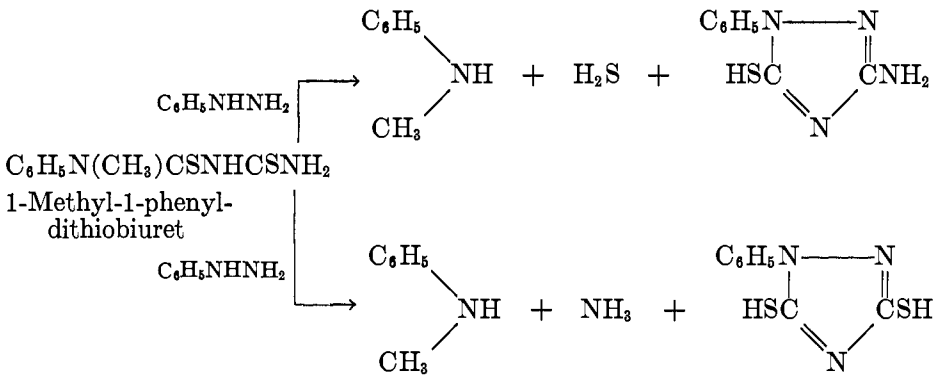
1,5-Dianilinodithiobiuret

(138, 140). The latter reaction yields a product which has been formulated as 1,5-dianilinodithiobiuret; its formation may be due to further interaction of the intermediate guanylthiourea with hydrogen sulfide, which arises in turn by the action of an excess of phenylhydrazine upon the liberated sulfur.

The structure of 1,5-dianilinodithiobiuret has not been rigidly proved and is open to the objection that the product, unlike other dithiobiurets, resists oxidation. Under the influence of potassium hydroxide or hydrochloric acid it yields triazoles, possibly according to the following equations (138):



1-Methyl-1-phenyldithiobiuret itself undergoes analogous changes on treatment with phenylhydrazine, substituted triazoles being formed. In particular, the usual elimination of the methylphenylamino group is noteworthy (138). Analogous ring-closures occur when hydrazine hydrate is employed (*cf.* the corresponding reactions of 1-aryldithiobiurets) (139).



C. 1,3-DISUBSTITUTED DITHIOBIURETS

Only one member of this class of compounds appears to be on record. 3-Benzyl-1-ethyldithiobiuret was prepared by the addition of the elements of hydrogen sulfide to the corresponding 1,3-disubstituted-3-cyanothiourea; the reaction occurred with some difficulty (178).

D. 1,5-DISUBSTITUTED DITHIOBIURETS

Numerous 1,5-disubstituted dithiobiurets have been synthesized by the dealkylation of the corresponding *S*-alkyl thioethers (*cf.* Section XI,D). In

addition to the usual thiohydrolytic agents (e.g., 269), the use of hydrogen sulfide in pyridine–triethylamine has recently been found particularly advantageous. In this medium, thiohydrolysis usually proceeds rapidly at room temperature (112, 113).

Attempts to prepare 1,5-disubstituted dithiobiurets by the direct condensation of thioureas and isothiocyanates gave negative results (77); this reaction is only moderately successful in the preparation of the corresponding biuret series.

Alcoholic ammonia, in the presence of mercuric oxide, replaces both sulfur atoms of 1,5-substituted dithiobiurets by imino groups. The antimalarial compound 1-*p*-chlorophenyl-5-isopropyldiguanide (Paludrine) has been synthesized from the corresponding dithiobiuret by this method (77).

E. TRISUBSTITUTED DITHIOBIURETS

Synthesis from thiocarbamyl thiocyanates

Thiocarbamyl thiocyanates are obtainable from thiocarbamyl chlorides and potassium thiocyanate in absolute ethanol, or from lead dithiocarbamates and cyanogen bromide in benzene. They react with aniline at room temperature, with loss of thiocyanic acid, to form trisubstituted thioureas.

Near their melting points, however, thiocarbamyl thiocyanates isomerize to the isothiocyanates, which now condense with one molecule of aniline to yield a trisubstituted dithiobiuret; 1-methyl-1,5-diphenyl-2,4-dithiobiuret has been prepared by this route (65).

$$R_2NCSCl + KSCN \rightarrow R_2NCSSCN + KCl$$

$$(R_2NCSS)_2Pb + 2CNBr \rightarrow 2R_2NCSSCN + PbBr_2$$

$$R_2NCSSCN + 2C_6H_5NH_2 \rightarrow R_2NCSNHC_6H_5 + C_6H_5NH_2 \cdot HSCN$$

$$C_6H_5N(CH_3)CSSCN \rightarrow C_6H_5N(CH_3)CSNCS$$

$$C_6H_5N(CH_3)CSNCS + C_6H_5NH_2 \rightarrow C_6H_5N(CH_3)CSNHCSNHC_6H_5$$

1-Methyl-1,5-diphenyl-2,4-dithiobiuret

F. PENTASUBSTITUTED DITHIOBIURETS

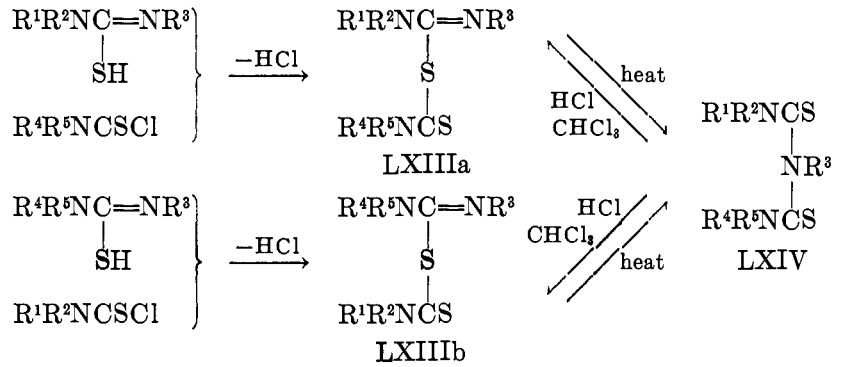
The interaction of disubstituted thiocarbamyl chlorides and primary amines normally affords 1,1,3-trisubstituted thioureas. By the use of an excess of thiocarbamyl chloride, both hydrogen atoms of the amine may be replaced and small proportions of fully substituted dithiobiurets obtained. Treatment of two

equivalents of ethylphenylthiocarbamyl chloride with aniline, for example, has given small yields of 1,5-diethyl-1,3,5-triphenyldithiobiuret (34).

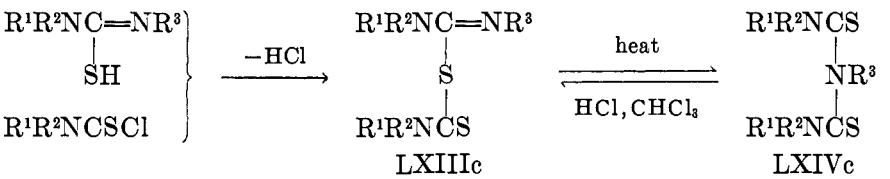
$$RR'NCSCl + R''NH_2 \rightarrow RR'NCSNHR'' + HCl$$

$$2RR'NCSCl + R''NH_2 \rightarrow RR'NCSNR''CSNRR' + 2HCl$$

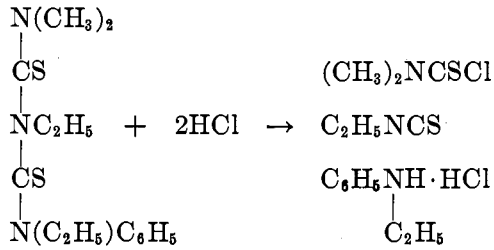
These observations suggested that equivalent quantities of 1,1,3-trisubstituted thioureas and thiocarbamyl chlorides should yield pentasubstituted dithiobiurets in the same way. Reaction occurred readily in chloroform but yielded in fact labile dithiocarbamides (LXIIIa and LXIIIb), which arose from the thiol configuration of the thioureas. Isomerization of these intermediates (LXIII) to dithiobiurets (LXIV) occurred instantly near their melting points, rapidly in boiling ethanolic solution or more slowly on storage. It was reversed by treatment with hydrogen chloride in chloroform (31, 33, 34). Interaction of the reagents by simple fusion, or in pyridine, led directly to dithiobiurets (LXIV), without the production of intermediate dithiocarbamides (31).



Billeter's interpretation of these results finds support in the experimental fact that the two isomeric intermediates (LXIIIa and LXIIIb), synthesized from suitable substituted starting materials (*cf.* above reaction scheme), yield the same dithiobiuret (LXIV) on isomerization. Conversely, the action of hydrogen chloride upon the dithiobiurets (LXIV: R^3 = aryl), in reversing the isomerization, produces mixtures of the two intermediate dithiocarbamides (in some, but not in all the cases studied). In the synthesis of symmetrically substituted dithiobiurets by this method, the complication of the existence of two isomeric intermediate dithiocarbamide bases (LXIII) does not, of course, arise (31, 33, 34; see also 63).



The isomerization of dithiobiurets to dithiocarbamates (LXIV → LXIII) under the influence of hydrogen chloride succeeds only with 3-aryl-substituted homologs; 3-alkyl derivatives yield products which suggest the occurrence of the following primary cleavage (33):



Pentasubstituted dithiobiurets are generally yellow crystalline solids, stable towards aqueous hydrochloric acid but readily isomerized by hydrogen chloride in chloroform solution (see above) (33). A comparison of the ultraviolet spectrum of pentamethyldithiobiuret with those of fully substituted thioureas has been made (320).

XI. Isodithiobiurets

Isodithiobiurets are available by the direct alkylation of dithiobiurets which, in common with thioureas, are attacked preferentially at the sulfur atoms in many of their reactions. The most general method of preparing isodithiobiurets is the extension of the Stieglitz–McKee synthesis of isobiurets, employing appropriately substituted isothioureas and isothiocyanates as reactants.

A. 1,2-disubstituted-2-isodithiobiurets

Isodithiobiurets of this type were first obtained by Tursini (404) by treatment of aromatic dithiobiurets with an alkyl iodide in the presence of ammonia. The linking of the alkyl group to a sulfur atom was clearly shown by the thermal decomposition of the products to thiols; their formulation as 1-aryl-2-alkyl-2-isodithiobiurets followed only later from their non-identity with 1-aryl-4-alkyl-4-isodithiobiurets, subsequently obtained by Johnson's unequivocal synthesis (*cf.* following section) (77, 209; compare also 146, 379).

$$RNHCSNHCSNH_2 + C_2H_5I + NH_3 \rightarrow RN{=}C(SC_2H_5)NHCSNH_2 + NH_4I$$

B. 1,4-disubstituted-4-isodithiobiurets

The condensation reactions between isoureas and isocyanates, developed by McKee (245), occur with equal facility in the sulfur series. Thus, 2-alkyl-2-isothioureas and isothiocyanates react smoothly in aqueous suspension at room temperature to produce good yields of the required products (210; see also 47, 407).

$$RNCS + NH_2C(SR'){=}NH \rightarrow RNHCSNHC(SR'){=}NH$$

Numerous 1-substituted dithiobiurets have been prepared by the hydrolytic removal of the alkyl groups from their enolic homologs. This thiohydrolysis is readily effected by the passage of hydrogen sulfide through their ethanolic solutions containing potassium hydrogen sulfide or sodium ethoxide (47, 407).

Ammonia, or primary amines, in alcoholic solution replace the alkylthiol grouping of 1,4-disubstituted-4-isodithiobiurets, with the formation of substituted guanylthioureas (77).

$$RNHCSNHC(SR')=NH + NH_2R'' \rightarrow RNHCSNHC(NHR'')=NH + R'SH$$

c. 1,2,4-trisubstituted-2,4-diisodithiobiurets

The alkylation of 1-aryldithiobiurets by an excess of dialkyl sulfate, or alkyl halide in ammoniacal ethanol, substitutes both thiol residues. Benzyl chloride, however, behaves anomalously (146, 379). The same products result, of course, when either 2- or 4-thioethers of 1-substituted dithiobiurets are further alkylated (379).

This property of dithiobiurets has proved useful in the preparation of homologs incorporating quaternary ammonium groups. The dithiobiuret residue of the unquaternized compound is first protected by conversion into its dithioether; the basic center can then be quaternized, and the protecting groups finally removed by thiohydrolysis (113).

d. 1,2,5-trisubstituted-2-isodithiobiurets

1,2-Disubstituted-2-isothioureas, readily available from arylthioureas and alkyl iodides, react smoothly with isothiocyanates in ethereal solution in the expected manner. More highly substituted isodithiobiurets result even more readily from 1,1,2-trisubstituted-2-isothioureas (77, 78, 210, 211, 212, 407).

$$RNHC(SR')=NH + R''NCS \rightarrow RN=C(SR')NHCSNHR''$$

LXV

$$R^1R^2NC(SR')=NH + R''NCS \rightarrow R^1R^2NC(SR')=NCSNHR''$$

The preferential addition of the isothiocyanate at the unsubstituted imino residue of the isothiourea is proved by the hydrolytic decomposition of the resulting isodithiobiurets (LXV) by hydrochloric acid at 100°C. The observed formation of aniline and thioethyl 4-phenyl-3-thioallophanate excludes the alternative possible structure (LXVI) (211, 212).

$$C_6H_5NHCSNHC(SC_2H_5)=NC_6H_5 + H_2O \rightarrow$$

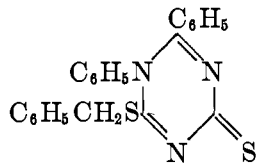
$$C_6H_5NH_2 + C_6H_5NHCSNHCOSC_2H_5$$

$$C_6H_5NHCSN(C_6H_5)C(SC_2H_5)=NH$$

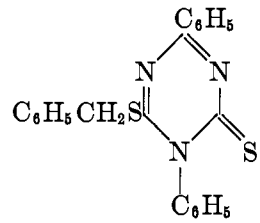
LXVI

With benzoyl isothiocyanate the usual reaction appears to be followed by immediate further changes. The violent interaction between 2-benzyl-1-phenyl-2-

isothiourea and benzoyl isothiocyanate, for example, does not yield the expected isodithiobiuret [$C_6H_5N{=}C(SCH_2C_6H_5)NHCSNHCOC_6H_5$], but affords a product probably formed therefrom by dehydrative cyclization, possibly of the triazine structure LXVII or LXVIII (212).



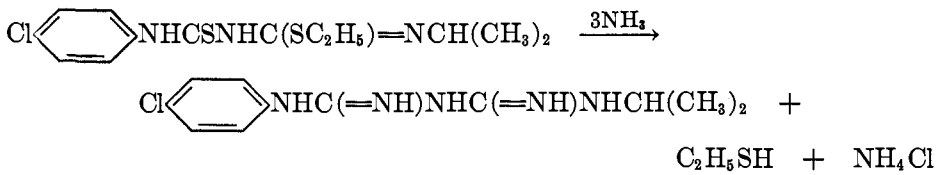
LXVII



LXVIII

Thiohydrolysis of the above isodithiobiurets affords 1,5-disubstituted dithiobiurets (407). The method has been applied successfully (77, 152) to the preparation of the dithiobiuret analog of the antimalarial compound Paludrine and of numerous other 1,5-disubstituted dithiobiurets (113).

In common with 1-aryldithiobiurets, isodithiobiurets of the above type are desulfurized by oxides of heavy metals, particularly mercuric oxide. In the presence of alcoholic ammonia the corresponding biguanides are formed (77, 78).



E. 1,1,2,5-TETRASUBSTITUTED-2-ISODITHIOBIURETS

1,1,2,5-Tetrasubstituted-2-isodithiobiurets, which are readily obtained by the general synthesis (*cf.* foregoing section), cannot be thiohydrolyzed to the normal dithiobiurets. Thus, Curd *et al.* (77) observed the complete breakdown of the appropriate isodithiobiurets in their attempts to prepare 1-(*p*-chlorophenyl)-5,5-dimethyl- and 1-(*p*-chlorophenyl)-5-methyl-5-isopropyldithiobiurets by this method.

F. 1,2,4,5-TETRASUBSTITUTED-2,4-DIISODITHIOBIURETS

These derivatives are rapidly formed by further alkylation of monothioethers of dithiobiurets; equivalent proportions of methyl iodide in ethanol or of dimethyl sulfate in acetone are suitable reagents (77, 210, 211, 212, 407).

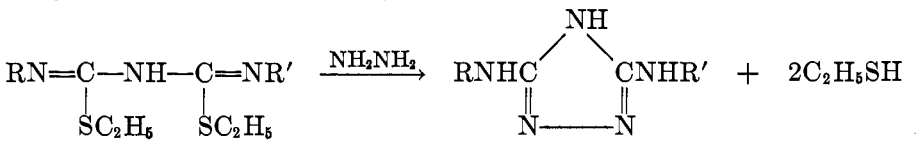
$$RN{=}C(SR')NHCSNHR + R'I \rightarrow RN{=}C(SR')NHC(SR'){=}NR \cdot HI$$

The fact that the alkyl radicals enter at the sulfur atom and not at a nitrogen atom follows directly from the observation that the same product results from

the methylation of 2-methyl-1-phenyl-5-*o*-tolyl-2-isodithiobiuret and 2-methyl-5-phenyl-1-*o*-tolyl-2-isodithiobiuret (209, 211).

$$C_6H_5N{=}C(SCH_3)NHCSNHC_6H_4CH_3\text{-}o$$
$$\searrow$$
$$C_6H_5N{=}C(SCH_3)NHC(SCH_3){=}NC_6H_4CH_3\text{-}o$$
$$\nearrow$$
$$o\text{-}CH_3C_6H_4N{=}C(SCH_3)NHCSNHC_6H_5$$

Isodithiobiurets of this class are cyclized by hydrazine hydrate to 3,5-dialkyl-(or aryl)amino-1,2,4-triazoles (77, 407).



XII. Acylbiurets and Analogs

A. Acylbiurets

In addition to the direct acylation of biuret, several alternative syntheses of acylbiurets have been developed. Since only a few acylated sulfur analogs have been reported, they are included in the general discussion below. No sulfonylbiurets have so far been described; reactions which might be expected to yield these derivatives proceed differently. In contrast, a series of *N*-sulfonyl-2,4-diisodithiobiurets has been prepared by the conventional method employing sulfonyl chlorides.

1. Synthesis

(a) Direct acylation

The acylation of biuret was first studied by Ostrogovich. Interaction with acetyl, benzoyl, or phenacetyl chloride in closed vessels above 100°C. afforded 65–75 per cent yields of the respective 1-acylbiurets (271, 272, 274, 277). More complex acyl chlorides (e.g., RR'CHCOCl) afford only low yields of acylated products, but such compounds are available by a superior alternative route (*cf.* Section XII,A,1(b)).

1-Alkylbiurets yield monoacetyl derivatives (40); their formulation as 1-acyl-1-alkylbiurets (69) is not established beyond doubt. 3-Methyl-, 3-ethyl-, and 1,5-dimethylbiurets similarly yield 1(?)-acetyl derivatives (40), but monoacyl derivatives of 1-arylbiurets have been represented as 1,5-disubstituted biurets (346).

While attempting to benzoylate urea in pyridine at 100°C., Walther and Wlodkowski (415) unexpectedly isolated 1-benzoylbiuret upon subsequent treatment of the crude product with glacial acetic acid. A detailed examination of this reaction by Ostrogovich (273) showed that, contrary to the opinion of Walther and Wlodkowski, 1-benzoylurea is not the primary intermediate product in this reaction, since 1-benzoylbiuret is not obtainable by refluxing authentic 1-benzoylurea in glacial acetic acid. On the other hand, there seems

to be some evidence for the assumption of a primary condensation of urea to biuret: thus, Bloch and Sobotka (51) have improved the method by first refluxing urea in pyridine for 2 hr. and then treating the resulting solution with benzoyl chloride. In all cases considerable quantities of benzoylurea, benzamide, and dibenzamide are formed in parallel reactions, and, in view of the difficulties of separating (51, 273) the complex mixtures, the practical usefulness of this method is limited.

The use of an acid amide as acylating agent has also been reported. Thus, 1,5-dianthranoylbiuret has been obtained almost quantitatively by heating biuret with 4 moles of anthranilamide to 140–145°C. (205).

$$2RCONH_2 + (NH_2CO)_2NH \rightarrow (RCONHCO)_2NH + 2NH_3$$

The acetylation of 1-aryldithiobiurets by acetyl chloride (404) or acetic anhydride (145) yields monoacetyl and diacetyl derivatives of unknown constitution.

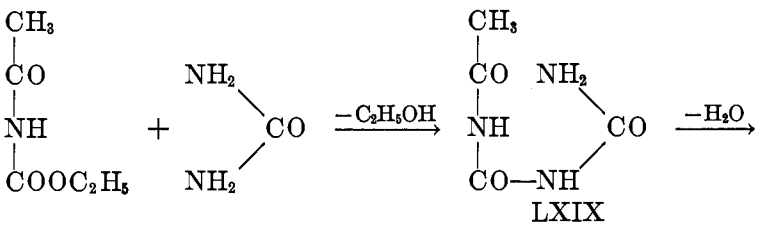
(b) From acyl isocyanates

Benzoyl, phenacetyl, and dialkylacetyl isocyanates, prepared from the appropriate acid chloride and silver cyanate (19, 32, 188), react with urea, or its 1-alkyl or 1,1-dialkyl derivatives, in boiling anhydrous ether or light petroleum to yield a wide range of substituted acylbiurets. With thiourea, 1-dialkylacetyl-4-thiobiurets are formed, but the yields are poor and the products are ill defined (188, 189, 190, 191). *p*-Nitrobenzoyl isocyanate similarly affords 1-(*p*-nitrobenzoyl)biuret, which, after reduction to the amino derivative, serves as source of the corresponding 1-*p*-arseno- and 1-*p*-arsenosobenzoylbiurets (370).

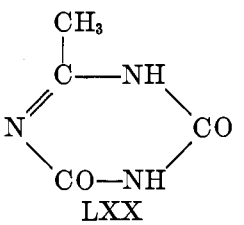
$$RR'CHCONCO + NH_2CONR''R''' \rightarrow RR'CHCONHCONHCONR''R'''$$

(c) From acylurethan and urethan

Acetylurethan and urea react at 150°C. in closed vessels to yield mainly 2-methyl-4,6-dioxy-*s*-triazine (LXX) (270), varying quantities of 1-acetylurea



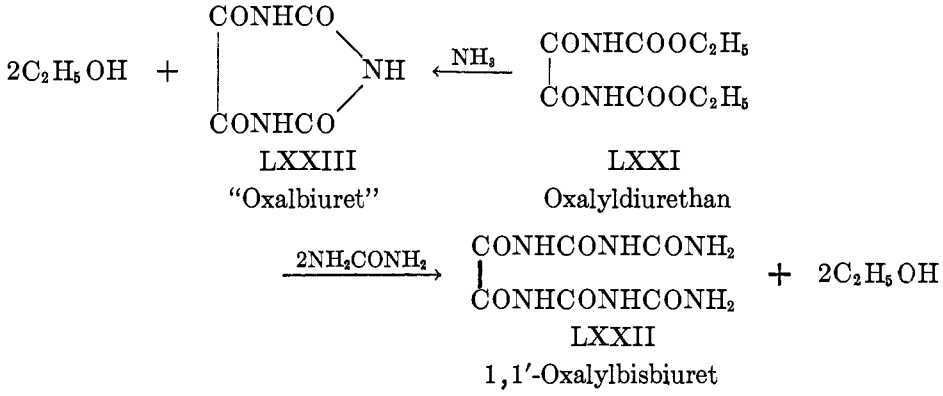
LXIX
Acetylbiuret



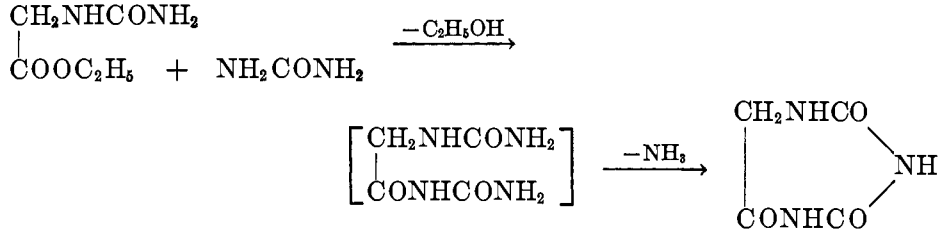
LXX

and 1-acetylbiuret being obtained as by-products; the same reaction occurs between acetylurea and urethan at 180°C. Acetylbiuret appears to be the primary product, which is subsequently cyclized to the triazine. This view finds support from the observation that 1-acetylbiuret itself is dehydrated to LXX on further treatment with acetyl chloride (275, 276).

A comparable synthesis is the formation of 1,1′-oxalylbisbiuret (LXXII), amongst other products, in the fusion of oxalyldiurethan (LXXI) and two molecules of urea at 135–140°C. Ammonolysis of oxalyldiurethan (LXXI) does not yield the expected oxalylbisurea, but continues, with elimination of ethanol, to furnish the cyclic "oxalbiuret" (LXXIII) (169).



Finally, minute yields of a compound formulated as a cyclic glycolylbiuret have been claimed to result from the fusion of hydantoic ester and urea at 130°C. (169).



(d) From allophanyl chloride

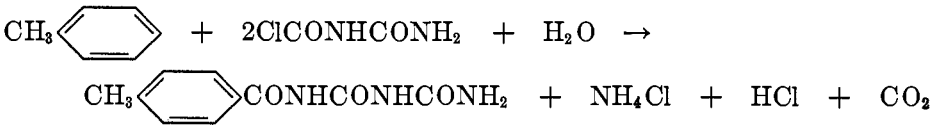
The condensation of allophanyl chloride with acid amides is a convenient method of preparing 1-acylbiurets. The reactants are heated in chlorobenzene solution until no more hydrogen chloride is evolved (202, 204).

$$RCONH_2 + ClCONHCONH_2 \rightarrow RCONHCONHCONH_2 + HCl$$

Allophanyl chloride

The interaction of allophanyl chloride and aromatic hydrocarbons in the presence of catalysts such as aluminum chloride, ferric chloride, or boron tri-

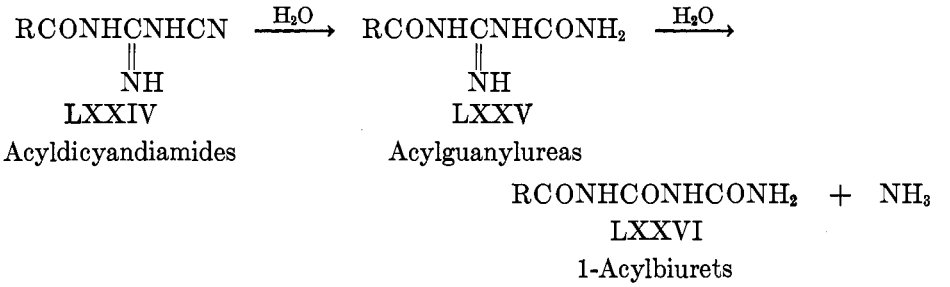
fluoride has been claimed to yield 1-aroylbiurets, the reaction being represented as follows (201, 203):



It may be noted at this point that attempts to prepare 1-acetylbiuret and 1-benzoylbiuret by the ammonolysis of ethyl 4-acylallophanates were unsuccessful. At 40–50°C. the reactant is merely hydrolyzed to ethyl allophanate; at 100°C. biuret and acetamide are formed (271).

(e) From acyldicyandiamides and acylguanylureas

The hydrolysis of dicyandiamide to guanylurea is a well-known reaction. Further hydrolytic action does not afford biuret; instead, cleavage of the molecule occurs and guanidine salts are formed (2, 126). The stepwise conversion of acyldicyandiamides (1-acyl-3-cyanoguanidines) (LXXIV) to acylguanylureas (LXXV) and thence to 1-acylbiurets (LXXVI), on the other hand, is readily accomplished by acid hydrolysis. The first stage is generally completed within a few minutes, but subsequent formation of the biuret proceeds considerably less rapidly; it is thus possible to isolate the intermediate guanylureas (LXXV) if desired. Most acids having dissociation constants greater than 10^{-4} and certain acidic salts (including cyanamide hydrochloride) are suitable hydrolytic agents. The hydrochloride of benzoylguanylurea is in fact hydrolyzed to the corresponding biuret in hot aqueous solution, no further acid being required (1, 2, 9).



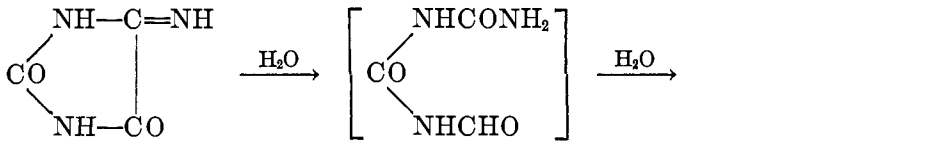
1-Acyl-3-cyanoguanidines (LXXIV), required as starting materials in this synthesis, are readily formed from dicyandiamide and the appropriate acyl anhydride or chloride in aqueous alkalies, preferably in the presence of organic diluents such as acetone (1, 2, 9, 215, 216).

The acid hydrolysis of acylguanylthioureas, obtained by the addition of the elements of hydrogen sulfide to acyldicyandiamides, has also been claimed to yield acylmonothiobiuret, but no examples or experimental details have been given (367).

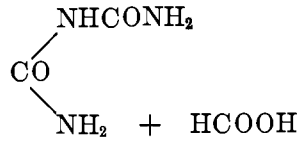
(f) From heterocyclic compounds

The facile cleavage of allantoxaidine across the 4,5-bond to biuret derivatives (*cf.* Sections II,B,10; III,A,1; V,A,1(m)) may be, by a proper choice of the hydro-

lytic agent, yield acylbiurets. Thus, dilute acids or boiling water afford biuret and formic acid, which arise probably from the unstable intermediate formylbiuret.



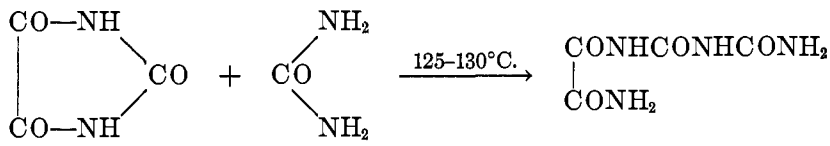
Allantoxaidine Formylbiuret



Boiling acetic anhydride, on the other hand, yields a stable formylacetylbiuret (in which the substituents occupy probably the 1- and 5-positions) (37). Chromic acid oxidation of 7,9-dimethyl-, or 4,7,9-trimethyl-4,5-dihydrouric acid yields, in addition to dimethylparabanic acid, a product believed to be 1-methyl-5-formylbiuret (36).

(g) Other reactions

The condensation of parabanic acid and urea at 125–130°C. affords a product which was formulated as 1-oxalylbiuret by its discoverer (165), but doubts concerning the true nature of this product have not been completely resolved (45, 304).



Parabanic acid

An unequivocal synthesis of 1-oxalylbiuret, by the ammonolysis of 1-carbethoxy-3-oxalylethoxyurea, appears to support Grimaux's original interpretation, but a careful comparison of the products is rendered difficult by their insolubility and their unfavorable physical properties (54, 55, 56).

$$C_2H_5OOCCONHCONHCOOC_2H_5 + 2NH_3 \rightarrow$$

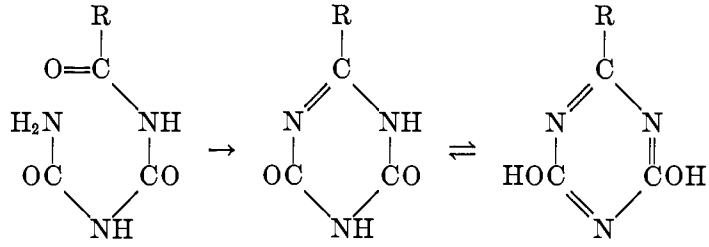
$$NH_2COCONHCONHCONH_2 + 2C_2H_5OH$$

2. Chemical properties

The lowest homologs, including 1-acylbiuret, are freely soluble in water and readily recrystallized therefrom; aroyl derivatives are usually purified from organic solvents (271). Crystallographic details concerning 1-benzoylbiuret, which crystallizes in the monoclinic system, are available (273).

Acylbiurets are generally soluble in alkalies and reprecipitated therefrom by dilute mineral acids (1). They are slowly hydrolyzed in boiling aqueous or alcoholic solution; decomposition occurs rapidly in dilute hydrochloric acid (271).

1-Acetylbiuret yields addition compounds containing one molecule of potassium or sodium hydroxide, or sodium ethoxide, respectively. They are precipitated as crystalline solids on mixing equivalent quantities of the components in saturated ethanolic solution (275).



Acylbiurets are readily cyclized, with loss of water, to 2-substituted-4,6-dihydroxy-1,3,5-triazines. The reaction is effected by acetyl chloride (276) and occurs rapidly and quantitatively in the presence of alkalies, preferably in the form of concentrated aqueous solutions (2, 9, 51, 202, 203, 204, 272, 277). The resulting products are useful sources of triazine vat dyes (9). The analogous cyclization of dithiobiuret, by successive treatment with benzoyl chloride and pyridine, yields 2-phenyl-4,6-dithiol-1,3,5-triazine (114).

The chlorination of 1-acetylbiuret in aqueous solution yields a disubstitution product to which the 1-acetyl-5,5-dichlorobiuret structure has been assigned (without evidence); 1-benzoylbiuret resists halogenation under the same conditions (88).

Like other 1-substituted biurets, 1-acylbiurets do not give the biuret reaction (271).

B. N-CARBOXYBIURETS

1. 1-Carbethoxybiuret

Dicarbethoxyurea, easily obtainable from phosgene and urethan, reacts with ammonia in two ways, according to the conditions. Liquid ammonia containing 1–2 per cent of water replaces one ethoxy group by an amino group and readily yields carbethoxybiuret as the main product.

$$\underset{}{COCl_2} + \underset{\text{Urethan}}{2NH_2COOC_2H_5} \rightarrow \underset{\text{Dicarbethoxyurea}}{C_2H_5OOCNHCONHCOOC_2H_5} \xrightarrow{NH_3}$$

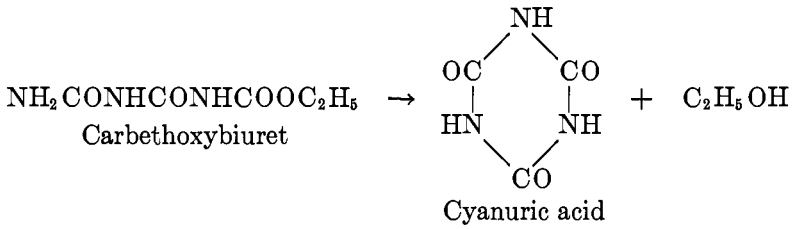
$$\underset{\text{Carbethoxybiuret}}{NH_2CONHCONHCOOC_2H_5} + C_2H_5OH$$

In concentrated aqueous ammonia, however, a carbethoxy group is removed hydrolytically, resulting in ethyl allophanate and cyanuric acid, the latter arising presumably from some primarily formed carbethoxybiuret (see below) (82).

The condensation of allophanyl chloride and urethan in chlorobenzene also yields the expected carbethoxybiuret (202).

$$\underset{\text{Urethan}}{C_2H_5OCONH_2} + \underset{\text{Allophanyl chloride}}{ClCONHCONH_2} \rightarrow \underset{\text{Carbethoxybiuret}}{C_2H_5OOCNHCONHCONH_2}$$

On dissolution in alkali, carbethoxybiuret is rapidly cyclized, with elimination of ethanol, to cyanuric acid (82):



2. 1-Aryl-5-carbethoxybiurets

The analogous reaction of dicarbethoxyurea with aniline, in the temperature range of 110–115°C., yields 1,5-diphenylbiuret and 5-carbethoxy-1-phenylbiuret; at higher temperatures (130–150°C.) 1,7-diphenyltriuret is also formed. Analogous observations with other arylamines in various temperature ranges suggest that the main reaction consists in the successive amination of the two carbethoxy groups (82).

$$CO(NHCOOC_2H_5)_2 + RNH_2 \rightarrow RNHCONHCONHCOOC_2H_5 + C_2H_5OH$$

$$CO(NHCOOC_2H_5)_2 + 2RNH_2 \rightarrow CO(NHCONHR)_2 + 2C_2H_5OH$$

Dicarbethoxyurea

In marked contrast, the sulfur atom is the point of attack in the corresponding reaction of dicarbethoxythiourea and its *S*-methyl ether; formation of dicarbethoxyguanidines occurs smoothly (261).

$$CS(NHCOOC_2H_5)_2 + RNH_2 \rightarrow RN{=}C(NHCOOC_2H_5)_2 + H_2S$$

Dicarbethoxythiourea

The reaction between 1 mole of phenyl isocyanate and 2 moles of urethan or ethyl allophanate at 130°C. during several hours results in the successive formation of the following series of compounds: phenylurethan, ethyl phenylallophanate, 5-carbethoxy-1-phenylbiuret, and phenylcyanuric acid (82, 261).

1-Aryl-5-carbethoxybiurets yield stable silver and sodium salts, the latter being obtainable under anhydrous conditions only. Like carbethoxybiuret, its aryl homologs are cyclized to *N*-arylcyanuric acids in aqueous alkaline solution (82).

3. Biuret-1-carboxylic acid

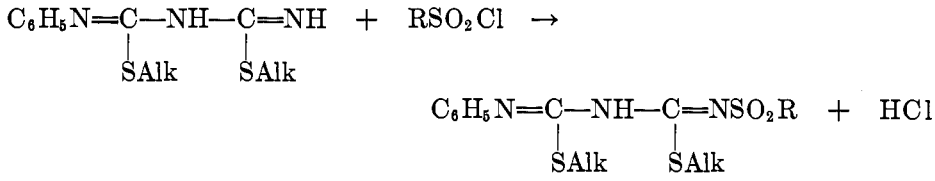
The production of the acid chloride of a biuretcarboxylic acid has been claimed (409) to have been achieved by heating carbamyl chloride by itself, or in inert solvents, until the appropriate loss of hydrogen chloride has taken place. The structure ($NH_2CONHCONHCOCl$) of the product follows from its hydrolysis by water to biuret and carbon dioxide.

C. SULFONYLBIURETS

N-Sulfonylbiurets have apparently so far not been described. The hydrolysis of *N*-arylsulfonyldicyandiamides, like that of dicyandiamide itself, does not

yield the corresponding biurets. With moderately concentrated aqueous-ethanolic sulfuric acid, the reaction proceeds only to the guanylurea stage; under more vigorous conditions the molecule is ruptured, an arylsulfonamide being formed as one of the main products (234). The action of sulfonyl chlorides on 1-aryl-biurets in pyridine yields chiefly trisulfonylmelamines (*cf.* Section V,A,2).

1,2,4-Trisubstituted-2,4-diisodithiobiurets are sufficiently strong bases to react readily with sulfonyl halides in acetone–pyridine. Since the thiol groups of the reactant are blocked, the resulting products are represented as *N*-sulfonyl derivatives (R = $CH_3CONHC_6H_4$—) (379).



XIII. Triurets

A. triuret (carbonic acid diureide; 1,3-di(aminoformyl)urea; carbonyldiurea; 1,3-dicarbamylurea)

Triuret, the compound which bears the same relationship to biuret as biuret does to urea, was first prepared in 1872. The generic term "triuret," the basis of the nomenclature most generally adopted for compounds of this structure, expresses their formal derivation from three molecules of urea (with loss of two molecules of ammonia). The mode of numbering the triuret structure used by *Chemical Abstracts* is shown in formula LXXVII.

$$\overset{1}{N}H_2\overset{2}{C}O\overset{3}{N}H\overset{4}{C}O\overset{5}{N}H\overset{6}{C}O\overset{7}{N}H_2$$

LXXVII

Triuret

1. Synthesis

The discovery of triuret is due to Schmidt (340), who first prepared this compound in 1872 by heating urea with an excess of phosgene under pressure at 100°C. Quantitative yields were also obtained when a 20 per cent solution of phosgene in toluene was employed (332; compare also 14). The interaction of oxamide and phosgene in closed vessels above 170°C. proceeded with similar results (340).

$$2NH_2CONH_2 + COCl_2 \rightarrow CO(NHCONH_2)_2 + 2HCl$$

$$\underset{\text{Oxamide}}{2(CONH_2)_2} + COCl_2 \rightarrow \underset{\text{Triuret}}{CO(NHCONH_2)_2} + 2CO + 2HCl$$

The deamination of urea by thionyl chloride yields, under proper conditions, either biuret or triuret as the main product and is claimed to be the most convenient method of preparing the latter compound, affording up to 30 per cent

yields. Traces of triuret are also obtained when sulfuryl chloride or chlorosulfonic acid is employed. In contrast, thiourea is not deaminated under similar conditions; it fails to react with boiling thionyl chloride or sulfuryl chloride and is decomposed, with liberation of sulfur, by chlorosulfonic acid (177, 420).

Some triuret is formed as a by-product, along with biuret, in the thermal deamination of urea (363), in the reaction of allophanyl chloride with ammonia (57), and in the interaction of urea and fumaric acid at 135–140°C. for 2.5 hr. (207).

Triuret is the product of the decomposition of acetylhydroxyoxamide (337) by warm aqueous sodium carbonate, probably by the following mechanism (*cf.* Section V,A,1(f)) (290):

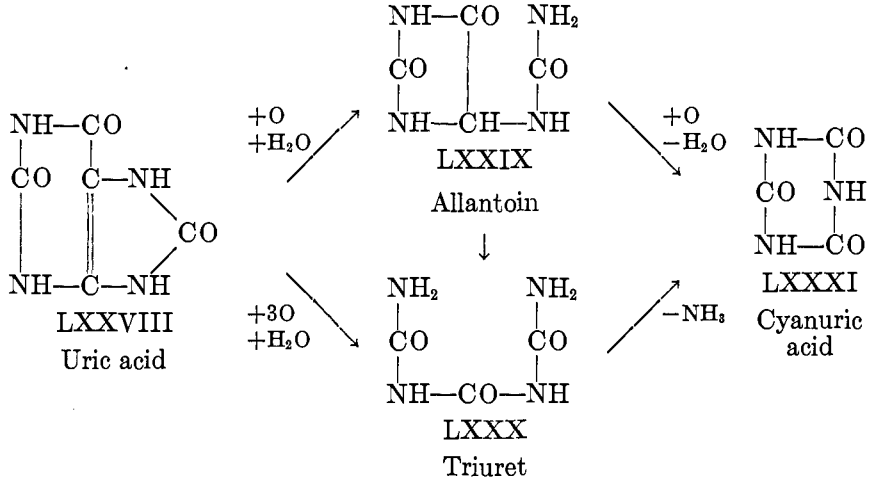
$$NH_2COCONHOCOCH_3 \rightarrow CH_3COOH + [NH_2CONCO]$$

$$2[NH_2CONCO] + H_2O \rightarrow (NH_2CONH)_2CO + CO_2$$

Triuret

Under carefully controlled conditions, triuret is found amongst the oxidative degradation products of certain purines.

The oxidation of uric acid (LXXVIII) by alkaline hydrogen peroxide affords, in addition to carbonic acid, ammonia, and occasional small quantities of urea and oxalic acid, three main products: cyanuric acid, triuret, and allantoin. The production of minute yields of triuret in caustic soda solution, first noticed by Schittenhelm and Wiener (339) and confirmed by Walters and Wise (412), was investigated systematically by Venable (408) with special reference to temperature and alkalinity. The results suggested that allantoin (LXXIX) and triuret (LXXX) (maximum yield, 18 per cent) were formed side by side in a parallel reaction, and were each further changed into cyanuric acid (LXXXI) subsequently.



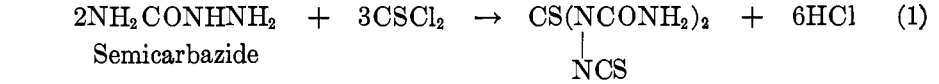
Contrary to Venable's views, some evidence has later been adduced for the successive oxidation of uric acid, via allantoin, to triuret; the oxidative con-

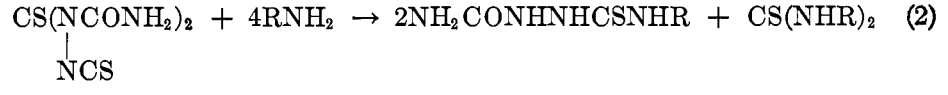
version of LXXIX into LXXX has subsequently been found to proceed, if only to a very limited extent, in the presence of potassium hydroxide (338) or, more efficiently, by electrochemical oxidation (117) (see below).

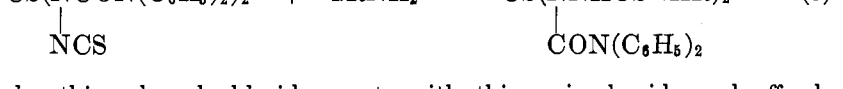
Triuret results in somewhat improved yields as the main product of the hydrogen peroxide oxidation of uric acid in water, in dilute acetic acid solution (23 per cent) (44), or in boiling 5 per cent ammonia (28 per cent) (338). Similar results are observed when boiling aqueous solutions of uric acid are treated with hydrogen peroxide in the presence of ferric chloride (267).

In their systematic work on the electrochemical oxidation of purines, Fichter and Kern (117) were able to convert both uric acid (LXXVIII) and allantoin (LXXIX) into triuret. Alkaline solutions of uric acid containing lithium carbonate yield allantoin (68.5 per cent) after a current consumption equivalent to one atom of oxygen (lead dioxide anode; current density, 0.01–0.05 amp./sq. cm.). Continued oxidation, employing three atoms of oxygen, yields triuret (13.8 per cent) instead. This observation, together with the fact that allantoin also yields triuret (11.7 per cent) on anodic oxidation, suggests the intermediate formation of allantoin (LXXIX) in the oxidation of uric acid to triuret.

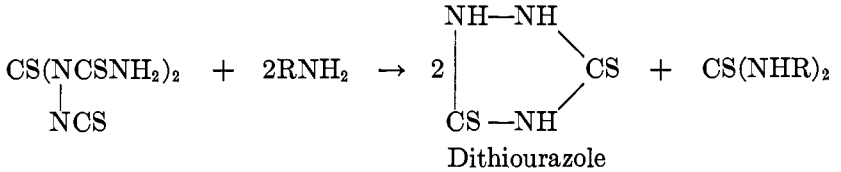
Reactions analogous to the general synthesis of triurets from ureas and phosgene have been carried out in the sulfur series (28). Semicarbazide hydrochloride, on treatment with an excess of thiocarbonyl chloride in aqueous suspension, yields 3,5-diisothiocyano-4-thiotriuret (equation 1), the structure of which follows from that of the analogous 1,1,7,7-tetraphenyl derivative (obtained from *N*,*N*-diphenylsemicarbazide) whose formation involves no structural ambiguities. Further support for the suggested structures of these two triurets is provided by their reaction with arylamines, which is found to proceed according to equations 2 and 3 (all the resulting compounds being identified) (28).





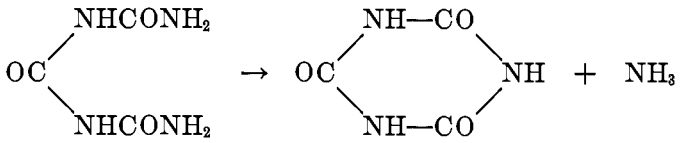


Similarly, thiocarbonyl chloride reacts with thiosemicarbazide and affords 3,5-diisothiocyano-2,4,6-trithiobiuret; this reacts with arylamines to form dithiourazole and a thiocarbanilide (28).



2. *Properties*

Triuret is a white crystalline solid which decomposes at its melting point (267, 332, 339, 412, 420); on careful thermal decomposition it yields cyanuric acid and ammonia, together with smaller amounts of ammonium cyanate, urea, biuret, and ammelide (340, 420).



Triuret is only very sparingly soluble in the usual organic solvents, but may readily be crystallized from large volumes of boiling water (340). It shows but a faint biuret reaction (332, 420).

In common with biuret, but unlike urea, triuret fails to yield salt-like addition products with acids in aqueous media (340).

Triuret dissolves readily in liquid ammonia and reacts with potassium amide in this medium to yield crystalline salt-like derivatives; according to the relative proportions of the reactants employed, monopotassium or dipotassium triuret is obtained (49).

$$C_3H_6O_3N_4 + KNH_2 \rightarrow C_3H_5O_3N_4K + NH_3$$

$$C_3H_6O_3N_4 + 2KNH_2 \rightarrow C_3H_4O_3N_4K_2 + 2NH_3$$

The alleged existence of an ammonium salt (81) has been disproved (49, 82) and that of a silver salt ($C_3H_4O_3N_4Ag_2$) (81, 339) is doubtful (82). An insoluble crystalline addition compound with mercuric oxide ($C_3H_6N_4O_3 \cdot HgO$) is precipitated quantitatively upon addition of mercuric nitrate to a hot aqueous solution of triuret; triuret may be regenerated from this product by treatment with hot dilute acids (340). An insoluble barium salt has also been described (339).

Triuret is unaffected on prolonged boiling with water or dilute ammonia (177, 338) and resists hydrolysis by hydrochloric acid. It dissolves in cold concentrated sulfuric acid without decomposition and may be recovered on dilution; hot sulfuric acid causes decomposition to carbon dioxide and ammonia. Nitric or nitrous acid also brings about hydrolysis at elevated temperatures (340).

$$CO(NHCONH_2)_2 + 3H_2O \rightarrow 3CO_2 + 4NH_3$$

$$CO(NHCONH_2)_2 + N_2O_3 \rightarrow 3CO_2 + 2NH_3 + 2N_2$$

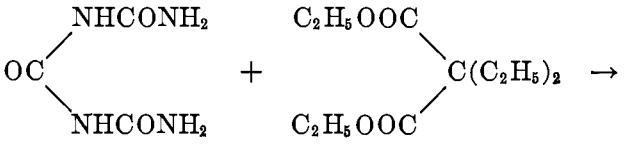
In 50 per cent sulfuric acid solution triuret reacts with nitrous acid, with the evolution of four equivalents of nitrogen, as follows:

$$CO(NHCONH_2)_2 + 4HNO_2 \rightarrow 4N_2 + 3CO_2 + 5H_2O$$

This behavior, resembling that of biuret, supports the constitution assigned to triuret (420).

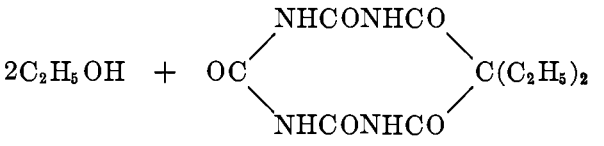
Triuret is soluble in alkalies and alkaline carbonates. On boiling, it is hydrolyzed to ammonia and cyanuric acid (340); the reaction occurs slowly at room temperature in 0.5 *N* sodium hydroxide, with or without the addition of hydrogen peroxide. In more weakly alkaline solution, hydrolysis proceeds very slowly even though the temperature be raised to 80°C. (408).

Treatment of triuret with phosgene at 150–160°C. in closed vessels yields mainly cyanuric acid (340). Interaction with substituted malonic esters in the presence of sodium, sodium amide, or sodium ethoxide is claimed to form cyclic products of the type of LXXXII (70).



Triuret

Diethyl diethylmalonate



LXXXII

A copper–triuret complex has been prepared and its effect on the rate of decomposition of hydrogen peroxide studied; the observed activity is much smaller than that of similar copper–biuret complexes (264).

B. SUBSTITUTED TRIURETS

Numerous triuret derivatives have been prepared by methods which are based either on the syntheses of the parent compound or on comparable routes to substituted biurets.

1. Synthesis

(a) From ureas

The formation of triuret from phosgene and urea (332, 340) can be extended to the preparation of substituted triurets. Methylurea, for example, affords 1,7-dimethyltriuret in 20 per cent yields (121), while 1-ethyl-1,3-diphenylthiourea, in the presence of pyridine, yields the symmetrical 2,6-dithiotriuret, $CO[N(C_6H_5)CSN(C_6H_5)C_2H_5]_2$ (83).

$$2RNHCONH_2 + COCl_2 \rightarrow CO(NHCONHR)_2 + 2HCl$$

With benzylideneureas the expected Schiff's bases of triuret are obtained. A series of substituted 1,7-dibenzylidenetriurets have been prepared in 60–70 per cent yields from benzylideneureas and phosgene, the products being isolated either directly or by way of the purified hydrochlorides. Like other biuret

derivatives containing the $C_6H_5CH{=}$ grouping, benzylidenetriurets do not give the biuret reaction (30).

$$2RCH{=}NCONH_2 + COCl_2 \rightarrow CO(NHCON{=}CHR)_2 + 2HCl$$

An analogous route leads to substituted dithiotriurets: interaction of phosgene in toluene with finely powdered sodium, potassium, or lead thiocyanate at 100°C. affords a solution containing carbonyl diisothiocyanate which reacts, *in situ*, with primary amines to form the expected thioureido derivatives. According to the nature and quantity of amine used, and to the experimental conditions employed, either *one* or *both* of the isothiocyanate groups are replaced. The intermediate substitution products, RNHCSNHCONCS, are sufficiently stable to be crystallizable from organic solvents; they are unaffected by cold water, but are cleaved by hot water into thiocyanic acid, arylthiourea, and carbon dioxide (100).

$$COCl_2 + Pb(SCN)_2 \rightarrow PbCl_2 + CO(NCS)_2$$

$$CO(NCS)_2 + RNH_2 \rightarrow RNHCSNHCONCS$$

$$CO(NCS)_2 + 2RNH_2 \rightarrow (RNHCSNH)_2CO$$

$$RNHCSNHCONCS + H_2O \rightarrow CO_2 + HCNS + RNHCSNH_2$$

(b) From dicarbethoxyurea

The reaction of dicarbethoxyurea with arylamines at fairly high temperatures yields (in addition to 1,5-diarylbiurets and 1-aryl-5-carbethoxybiurets; *cf.* Section XII,B,2) varying quantities of 1,7-diaryltriurets. The practical usefulness of this reaction appears to be limited by the extensive fractional crystallization necessary to isolate the individual products (82).

$$CO(NHCOOC_2H_5)_2 + 2RNH_2 \rightarrow CO(NHCONHR)_2 + 2C_2H_5OH$$

Dicarbethoxyurea

(c) From allophanyl chloride

The production of 1-phenyltriuret from allophanyl chloride and phenylurea in boiling chlorobenzene has been described in the patent literature (202).

$$C_6H_5NHCONH_2 + ClCONHCONH_2 \rightarrow C_6H_5NHCONHCONHCONH_2 + HCl$$

Phenylurea Allophanyl chloride 1-Phenyltriuret

(d) From hydroxyoxamides (*cf.* Section V,A,1(f) for fuller discussion)

Metallic salts of acetylhydroxyoxamides are decomposed in the presence of water to yield 1,7-disubstituted triurets. Combination of two molecules of the hypothetical intermediate substituted diisocyanic acid with water, with simultaneous evolution of carbon dioxide, accounts for the course of this reaction (289, 290).

$$RNHCOC(OK){=}NOCOCH_3 \rightarrow CH_3COOK + [RNHCONCO]$$

$$2[RNHCONCO] + H_2O \rightarrow CO_2 + (RNHCONH)_2CO$$

Some doubt concerning the correctness of this work arises from the fact that the physical constants of several diaryltriurets subsequently prepared by other syntheses (62, 82), though agreeing amongst themselves, differ from those obtained by the above hydroxyoxamide synthesis.

(e) From isotriurets

4-Methyl-1-phenyl-4-isobiuret, obtained from methylisourea and phenyl isocyanate (245), is capable of condensing with a second molecule of isocyanate. The 4-methyl-1,7-diphenyl-4-isotriuret thus formed, like all isoureas, is demethylated by hydrogen chloride to yield 1,7-diphenyltriuret (62).

S-Methyl-, *S*-ethyl-, and ω-ethoxyethylisothioureas react similarly, in the presence of alkali, with two molecules of phenyl isocyanate. The resulting isothiotriurets [the structure of which is further confirmed by their synthesis from 4-methyl-1-phenyl-4-isothiobiuret and isocyanate] are dealkylated by potassium hydrogen sulfide to 1,7-diphenyl-4-thiotriurets (236, 269). Isothiourea ethers, on the other hand, react with *one* molecule of isothiocyanate only, so that reaction does not proceed beyond the formation of the appropriate isothiobiuret (236).

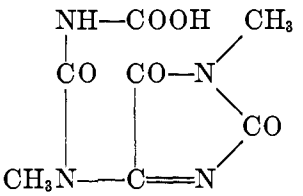
$$C_6H_5NHCONHC(OCH_3)=NH + C_6H_5NCO \rightarrow C_6H_5NHCONHC(OCH_3)=NCONHC_6H_5$$

$$C_6H_5NHCONHC(OCH_3)=NCONHC_6H_5 + HCl \rightarrow CH_3Cl + (C_6H_5NHCONH)_2CO$$

$$NH_2C(SR)=NH + 2C_6H_5NCO \rightarrow C_6H_5NHCONHC(SR)=NCONHC_6H_5$$

(f) From purines

Like biuret and triuret, a substituted triuret has been isolated from the degradation products of purines. Oxidation of theobromine by chlorine in chloroform yields an acidic product (LXXXIII) which is slowly decomposed by hydrochloric acid to 1,7-dimethyltriuret (121). The possible formulation of this triuret as the 1,5-isomer has been discussed by Biltz (39).



LXXXIII

2. Chemical properties

Little information concerning the chemical behavior of substituted triurets is available. 1,7-Dimethyltriuret is soluble in aqueous alkalies and is reprecipitated therefrom by the addition of mineral acids. Unlike triuret, it is not precipitated by mercuric nitrate (121). Boiling alkalies rapidly cyclize 1,7-dimethyltriuret,

with simultaneous elimination of methylamine, to *N*-methylcyanuric acid (39, 121). Like corresponding biurets, substituted triurets are capable of forming nitroso derivatives under the usual conditions; 1,7-dimethyltriuret yields a typical yellow derivative, for which the 1-nitroso structure has been suggested (121).

XIV. Tetruret and Carbonyldibiuret

There appears to be room for considerable doubt concerning the existence of the two higher analogs of biuret: namely, tetruret and carbonyldibiuret (420).

A. Tetruret, $NH(CONHCONH_2)_2$

The alleged syntheses of tetruret from allophanic azide and ammonia (383) or by the condensation of biuret and nitrobiuret (90) have not been confirmed. In subsequent work, only biuret was obtained in either of these reactions (242, 420).

B. Carbonyldibiuret, $CO(NHCONHCONH_2)_2$

In researches dealing with the action of phosgene on amides, Schmidt (340), by heating biuret with a large excess of phosgene in closed vessels at 60°C., obtained a product which agreed in composition with its formulation as carbonyldibiuret.

$$\underset{\text{Biuret}}{2NH_2CONHCONH_2} + COCl_2 \rightarrow \underset{\text{Carbonyldibiuret}}{CO(NHCONHCONH_2)_2} + 2HCl$$

The use of 20 per cent phosgene in toluene at 100°C. during 2 days afforded the same product in smaller yields (332).

In its solubility, its behavior on heating, and its action with acids and alkalies, carbonyldibiuret showed behavior almost identical with that of triuret. It gave an addition compound, $C_5H_8O_5N_6 \cdot 3HgO$, on treatment with mercuric nitrate (340) and showed a negative or feeble biuret reaction (332). On further reaction with phosgene under pressure at 140–150°C., carbonyldibiuret was completely decomposed into cyanuric acid and hydrogen chloride (340).

$$C_5H_8N_6O_5 + COCl_2 \rightarrow 2C_3H_3N_3O_3 + 2HCl$$

A comparison of some of the properties of biuret and its analogs (*cf.* table 8), together with other considerations, suggests strongly (420) that "tetruret"

TABLE 8

Comparison of some of the properties of biuret and its analogs

Compound	Melting Point (Decomposition)	Biuret Reaction	Solubility in Cold Water
	°C.		
Biuret	190	Positive	Soluble
Triuret	231	Negative or faint	Slightly soluble
Tetruret	186	Positive	Soluble
Carbonyldibiuret	235	Negative	Almost insoluble

may well have consisted of impure specimens of biuret, while the properties of "carbonyldibiuret" would be simulated by mixtures of triuret and cyanuric acid. The doubts concerning the existence of tetruret and carbonyldibiuret receive a further measure of support from the fact that no derivatives of either of these compounds have been prepared, whereas numerous derivatives of triuret are known (*cf.* Section XIII,B).

XV. Physiological and Pharmacological Properties

During recent years a considerable volume of research has dealt with various aspects of the biological properties of biuret and its derivatives. Although some hopes in the field of chemotherapy have been disappointed, certain biuret derivatives have been found to possess effective hypnotic, anticonvulsant, bacteriostatic, and cytotoxic properties. Others display marked insecticidal and rodenticidal powers, while their herbicidal effects should render them useful in the control of weeds. In the following section, brief reference is made to the publications concerned.

A. BIURET AND DERIVATIVES

The addition of biuret to pepsin increases the peptic activity of this proteoclastic enzyme (25). The enzymatic hydrolysis of biuret by Soya bean or gastric urease, reported by Takeuchi (380), has not been confirmed by later workers (244, 349). Data are available concerning the effect of biuret on the volume of red blood cells suspended in sodium chloride solution (106).

Biuret has no antipyretic action but causes a lowering of blood pressure (185). In experiments with rats, biuret showed diuretic properties but was strongly irritating to the urinary tract (170). This diuretic activity is only weak, as shown by the following comparison with a number of common diuretics (the activity of urea being taken as unity) (243):

Salyrgan	400	Theobromine	7.2
Theophylline	115	Ammonium chloride	2.7
Caffeine	32	Biuret	1.4

1-(Dialkylacetyl)biurets show pronounced hypnotic properties, comparing favorably with sodium barbital in hypnotic effectiveness. Their toxicity is remarkably low, doses of 500 mg./kg. having no permanent ill effect on test animals. The presence of branched chains in the acyl radical and of alkyl groups in the 5-position of the biuret skeleton increases the pharmacological action, 5,5-cyclopentamethylene-1-(diethylacetyl)biuret (and other related compounds incorporating the piperidine structure) being the most active of a series of biuret derivatives tested (188). 5,5-Cyclopentamethylene-1-diethylacetylbiuret possesses anticonvulsant activity in mice after the administration of lethal doses of cocaine, picrotoxin, and strychnine. The symmetrical 1,5-dipiperidyl derivative is a particularly effective antidote against picrotoxin. These biuret derivatives compare favorably, in both action and toxicity, with the commonly used hyp-

notics and anticonvulsants, including sodium pentobarbital and dilantin (11, 12, 13). Their effect in relaxing uterine muscle is only feeble (281).

Like other substances incorporating the —NHCO— system, biuret exhibits slight bacteriostatic effects, as well as synergistic effects with sulfanilamide, against *Trichophyton gypseum* (255). The synergistic action of sulfanilamide with biuret was also observed on *Escherichia coli* when grown on a synthetic medium containing *p*-aminobenzoic acid (350). Both bactericidal and bacteriostatic properties have been claimed for 1-(5-nitrofurfurylideneamino)biuret (373).

The cytotoxic effect of 1,5-bis(hydroxymethyl)biuret on the growth of Walker tumor transplants in rats has been studied (179).

The effect of biuret derivatives on plant growth has been the subject of a number of investigations. The unfavorable influence of small quantities of biuret (e.g., 0.01 per cent solutions) on chlorophyll-containing plants was demonstrated as early as 1904 (219). Concentrations of biuret necessary for the control of weeds, and the period of time that must elapse before other crops can safely be planted on treated plots, have been determined (128). When added as a herbicide to soil (0.25–0.5 g. per 1000 g. of soil), biuret does not appear to be harmful to any of the microörganisms (360). Data are available concerning the permeability of plant cells to biuret, determined by the plasmolytic method (429).

The growth-inhibiting effect of 1-phenylbiuret on plants, compared with that of (2,4-dichlorophenoxy)acetic acid by three standard tests, is low (385).

B. THIOBIURET AND DITHIOBIURET AND DERIVATIVES

Thiobiuret and its alkylated derivatives act as antipyretics when administered subcutaneously (to rabbits). Lethal doses cause decreased blood pressure, lung edema, and general collapse (185). Antipyretic effects are also produced (in rabbits) by 1-methyldithiobiuret in doses of 0.05 g./kg. of body weight (185). Dithiobiuret possesses anticonvulsant action but is effective only when toxic doses are given (7).

A detailed investigation concerning the bacteria-inhibiting action of 1-aryldithiobiurets against staphylococci, *B. coli*, pleuropneumonia organisms, and tubercle bacteria, carried out within the framework of a wide examination of compounds incorporating the $=N-\underset{|}{C}=S$ system, is due to Hagelloch and Liebermeister (172). 1-Aryldithiobiurets were found to possess good bacteriostatic properties, but proved to be rather toxic. Dithiobiuret itself is an inhibitor, if not the most effective one, for the growth of *Cryptococcus neoformans* in liquid media. This yeast-like organism causes the disease cryptococcosis, which is fatal to warm-blooded animals once the central nervous system is involved (341). Condensation products of dithiobiuret and aldehydes possess antitubercular activity *in vitro*. These derivatives, originally believed to be structural analogs of the well-known antitubercular compound Tibione ($CH_3CONHC_6H_4CH=NNHCSNH_2$) (127) have subsequently been proved to be triazine derivatives (114) (*cf.* Section X,A,2).

The trypanocidal activity of numerous dithiobiurets has been systematically investigated (435). Because of the low water-solubility of these compounds, their aqueous suspensions containing a dispersing agent were administered subcutaneously to mice. None of the derivatives tested showed appreciable trypanocidal activity against any species other than *Trypanosoma congolense*. 1-Aryldithiobiurets, particularly the 1-methyl-1-phenyl derivative, combined low toxicity with a reasonable activity after repeated doses. A number of more highly substituted dithiobiurets, as well as isodithiobiurets, were devoid of activity (435). Trypanocidal activity is frequently dependent on the presence of quaternary ammonium groupings. However, a number of dithiobiurets incorporating this structural feature did not show significantly higher trypanocidal activity than that originally observed in 1-phenyldithiobiuret (113).

A number of mono- and dithiobiurets structurally analogous to the antimalarial compound Paludrine have proved to be devoid of any antimalarial activity (77; see also 430).

Dithiobiuret shows slight antivesicant action and is moderately effective in the decontamination of Lewisite on human skin (122, 386).

A number of researches in plant physiology have dealt with certain biological properties of dithiobiuret. Solutions of dithiobiuret (20–80 mg./l.) in distilled water or phosphate buffer have a striking effect in developing roots on vine cuttings (105). When applied to the terminal bud of bean seedlings (*Phaseolus vulgaris*), dithiobiuret shows a moderate activity in inducing the formation of an abscission layer in the subjacent internode (418). In concentrations less than 10 mg./l. the compound has a depressing effect on the germination and on the growth of roots and coleoptiles of rice seedlings; concentrations of 10-25 mg./l., however, are highly beneficial in promoting seed germination (104, 105). It has been suggested that dithiobiuret may be used to delay the wilting of flowers; the favorable effect may be due to its protecting phenolic compounds against oxidation (105). The reducing properties of dithiobiuret have already been stressed: the compound operates as a hydrogen donor at hydrogen-ion concentrations within the biological range. Thus, for example, even dilute solutions (0.0005 *M*) are capable of rapidly decolorizing toluylene blue in media of pH not exceeding 7.5; the dye is only slowly reduced by cysteine (105). The stabilizing effect of dithiobiuret on the activity of amylase preparations has also been noted (411).

Toxicity

Dithiobiuret does not appear to present severe hazards by skin penetration. An aqueous paste of the compound held in contact with the skin of rabbits (1000 mg./kg.) during 18 hr. did not cause any obvious toxic effects (10).

Small concentrations of dithiobiuret are lethal within 7 days. Death is preceded by paralysis and involves presumably the respiratory muscles. A dose of 20–50 mg. kills an adult rat from a single injection, but fatal paralysis is caused within 5 days by daily administration of less than 0.5 mg., either in the drinking water or subcutaneously (16, 347). The paralysis is not accompanied by any significant changes in the water, chloride, sodium, potassium, phosphorus, or

creatine contents of skeletal muscle, or in the carbon dioxide, calcium, sodium, and potassium contents of the plasma (347).

Dithiobiuret and 1-phenyldithiobiuret were amongst one hundred ninety-six nitrogen-containing compounds examined for their rodenticidal properties against Norway rats, but, in common with all other products tested, did not exceed 1-naphthylthiourea (ANTU) in toxicity (96). The insecticidal properties of dithiobiurets [e.g., the 1,5-bis(hydroxymethyl) derivative] have been noted (366). 1-Phenyl- and 1-(2,4-xylyl)dithiobiuret are useful in combating the Japanese beetle, the iron salt of the former being as effective as lead arsenate. Dithiobiuret and its 1-(*p*-tolyl) derivative are potential control agents for the Mexican bean beetle (59).

C. TRIURET

After intravenous administration, triuret is contained largely unchanged in the urine (338) (dog, 78 per cent; man, 38–46 per cent). On oral administration it is oxidized to urea, ammonia, and carbon dioxide (180).

XVI. The Biuret Reaction

This brief section is not intended to review exhaustively what has become a specialized field with a voluminous literature. Attention is focussed on such work as has dealt directly with the ability of biuret and its derivatives to form colored coördination complexes. The extensive researches on the power of several classes of compounds other than biuret (particularly polypeptides and proteins) to show a "biuret reaction" have previously been summarized (225, 297) and are considered to be outside the scope of the present review, except in so far as they concern the structure of the biuret complexes. However, references have been included to all significant papers on this subject as guidance for the interested reader.

A. HISTORICAL

The characteristic reddish-violet color developed upon the addition of copper sulfate to biuret in the presence of alkali was first noted by Wiedemann in 1848, who discovered biuret as well as its distinctive color reaction at the same time (428). Hofmann (193) stressed the remarkable sensitivity of this test and suggested its suitability for the detection of small quantities of urea. It was soon observed (328) that the "biuret" color is caused by other soluble copper salts, and that strong alkalies are not essential to the success of the reaction; analogous colors are produced in the presence of weaker bases, including barium or calcium hydroxides, sodium carbonate, magnesium oxide, ammonium hydroxide, trialkylamines, or piperidine (328). The first systematic studies on the chemical nature of the colored substances responsible for the biuret reaction were carried out by Schiff (333, 335, 336) and by Tschugaeff (398–402) at the turn of the present century. Important recent contributions to the subject include the work of Traube (391–395) and of Pfeiffer (286, 287) and of their schools.

B. EXPERIMENTAL DETAILS

The biuret reaction is readily performed by the addition of caustic alkali and copper sulfate solution, in either sequence, to the biuret derivative. A satisfactory reagent may in fact be obtained by mixing 25 ml. of 3 per cent aqueous copper sulfate with 1 liter of 10 per cent sodium hydroxide solution (218). Efforts to prepare a colorless biuret reagent have not been wholly successful (218). A solution containing 0.1 g. of potassium cuprocyanide in 50 ml. of 10 per cent aqueous sodium hydroxide remains colorless during several hours (384); this freshly prepared reagent has been recommended for performing the biuret test (384). Directions for carrying out the biuret reaction under standard conditions are available (208, 221).

The colored solutions containing the biuret complex behave in accordance with the Beer–Lambert law: provided they are freshly prepared, under standard conditions, the quality of their violet color is always the same, and its depth, estimated by means of the Duboscq colorimeter, is directly proportional to the concentration. On storage, however, particularly in contact with an excess of cupric hydroxide, both the quality and intensity of the color undergo rapid changes (208). These observations are the basis of successful quantitative methods of estimating biuret (157, 208). A simple colorimetric determination, using Nessler cylinders, was employed by Werner (423).

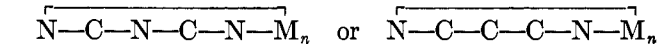
Following earlier measurements (222), Wokes and Still (433) examined the light-absorbing properties of solutions containing biuret–copper complexes in the wavelength range of 440 to 640 mμ and located the absorption maximum at 535 mμ. Their results agree essentially with a similar subsequent determination due to Poddubnaya and Gavrilov (302), who reported the absorption maximum at 530 mμ (molar extinction coefficient = 0.189). Very extensive spectrophotometric studies have recently been carried out, particularly by Plekhan, Gavrilov, and their coworkers, in connection with the "biuret colors" developed by polypeptides (159, 294, 298, 299, 323, 343) and proteins (158, 295, 296, 300, 306, 355).

Data are also available (64) concerning the paramagnetic properties of biuret–copper complexes.

C. THE SCOPE OF THE BIURET REACTION

Schiff's early investigations (333, 336) had shown that numerous substances other than biuret are capable of giving the characteristic violet biuret color, and that such compounds could be related to three fundamental structures: *viz.*, biuret, malonamide, and oxamide (333, 336). Further data which contributed to the knowledge of the biuret test were provided by the analogous behavior of certain acid amides (226, 313, 316, 333, 336, 348, 437), acid imides (238, 239, 314, 315, 398, 399, 400), and hydroxyamino derivatives (151, 387, 388, 389, 403). The discovery by Ritthausen (319) that proteins give a "biuret color" with the usual reagents has led to its practical application in protein chemistry; the reaction has been in common use as a sensitive test for polypeptides (195, 315, 390) and proteins (196, 265) for more than eighty years. This aspect of the subject has been reviewed by Plekhan and Gavrilov (297).

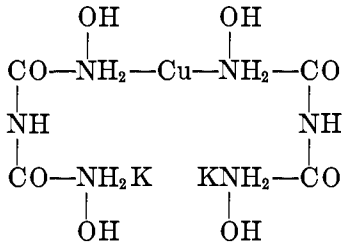
The general applicability of the biuret reaction to both biurets and other compounds has recently been summarized by Korenman (225), who classified such substances into thirteen structural systems. Their common, indispensable feature is their power to form six-membered internal complexes of the type



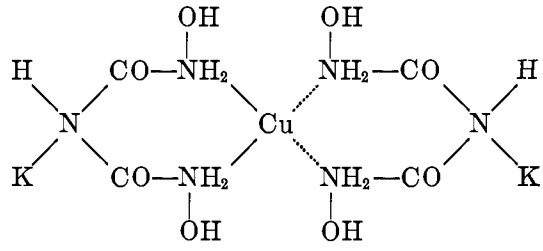
(where M is a metal and n an integer). The accumulated experience on the "biuret reaction" of the numerous and diverse types of structures has been valuable in interpreting complex formation involving biuret itself.

D. THE STRUCTURE OF BIURET–METAL COMPLEXES

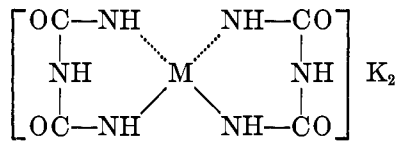
The simplest copper compound responsible for the violet color of the biuret reaction was first isolated in the pure state by Schiff (333, 336) in 1896. The composition of this product, which formed well-defined red leaflets or needles (240, 336, 395), was correctly determined. Its formation was shown to involve one atom of copper (or nickel; see below), two molecules of biuret, and two molecules of alkali, its molecular formula being given as $Cu(OH)_2 \cdot 2KOH \cdot 2C_2H_5N_3O_2$ (333, 336). Schiff also described the analogous behavior of nickel salts towards biuret; comparable products, but of intense yellow color, were isolated and analyzed (333, 336; see also 393–395 and table 11). The results were confirmed by later investigators (223, 240, 313), but in view of the inadequacy of contemporary valency theory, their interpretation in terms of structural formulas proved difficult. Early ideas concerning the nature of these products are expressed by structures such as LXXXIV (336) or LXXXV (223).



LXXXIV

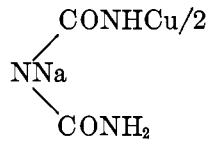


LXXXV



M = metal

LXXXVI



LXXXVII

On the basis of detailed researches into the power of imides and related compounds to form complexes with metals, Tschugaeff (400) proposed in 1907 to represent the analogous biuret derivatives as complex coördination compounds (LXXXVI) (see also 313). In its essentials, this formulation has been found the most satisfactory in later work.

TABLE 9

Molecular addition compounds of biuret

Compound	Reagent	Appearance	References
$C_2H_5N_3O_2 + NaOH$	Sodium hydroxide		(333, 336)
$C_2H_5N_3O_2 + KOH$	Potassium hydroxide	Needles	(333, 336)
$2C_2H_5N_3O_2 + CuSO_4$	Copper sulfate	Blue needles	(333, 336)
$2C_2H_5N_3O_2 + Cu(NO_3)_2$	Copper nitrate	Blue crystals	(333, 336)
$2C_2H_5N_3O_2 + CuCl_2$	Copper chloride	Green powder	(333, 336)
$C_2H_5N_3O_2 + HgO$	Mercuric nitrate	White powder	(333, 336)
$(C_2H_4N_3O_2)_2Hg + 2HgO$	Mercuric nitrate	Yellow flocculeS	(333, 336)
$2C_2H_5N_3O_2 + NiSO_4$	Nickel sulfate	Pale-green; crystalline	(336)
$2C_2H_5N_3O_2 + NiCl_2$	Nickel chloride	Pale-green; crystalline	(336)
$2C_2H_5N_3O_2 + CdCl_2$	Cadmium chloride	Crystalline; m.p. 255–260°C.	(329)
$C_2H_3N_3O_2Ag_2$	Silver nitrate and ammonia	White precipitate	(431)

Much precise information on the nature of these biuret coördination complexes was provided by the researches of Traube and his collaborators (391–395), who prepared and examined a large number of copper and nickel complexes and formulated Schiff's (333, 336) original compounds as $[(C_2H_3N_3O_2)_2Cu]K_2$ and $[(C_2H_3N_3O_2)_2Ni]K_2$, respectively (391, 395). These extensive experiments showed that the alkalies necessary for the production of the "biuret color" may be replaced by organic ammonium, sulfonium, and phosphonium hydroxides (395), as well as by ethylenediamine bases (395), resulting in red complex copper salts or yellow complex nickel salts of comparable structure (*cf.* tables 10 and 11). Thallium copper biuret and thallium nickel biuret differ from the majority of their analogs in being isolated without water of crystallization. Since these substances retain the usual brilliant red or yellow color, it may be concluded that the water of crystallization is not vitally concerned in their structure (393).

The color of the biuret copper or nickel complexes is associated with their complex anions; provided the cations are colorless, the salts possess, in the pure state, a pink to red or a yellow color. In marked contrast, however, biuret complexes prepared from cupric tetrammine or cupric ethylenediamine hydroxide are distinctly violet solids. This observation is readily accounted for by reference to the accepted formulation of such complexes (LXXXVIII and LXXXIX). The substances in question contain two atoms of copper, one of which is associated with the (red) complex anion, while the other forms part of the deep blue cupric tetrammine or ethylenediamine cation (391); the observed violet color of the resulting compounds appears to lend support to the general representation of biuret coördination complexes. Attention must be drawn, however, to Traube's discussion (394) of "inner salt" structures such as LXXXVII for these biuret derivatives.

$[(C_2H_3N_3O_2)_2Cu][Cu(en)_2]$ $[(C_2H_3N_3O_2)_2Cu][Cu(NH_3)_4]$

LXXXVIII LXXXIX

TABLE 10

Biuret-copper complexes

Compound	Reagent	Appearance	References
$[(C_2H_3N_3O_2)_2Cu]K$	Copper sulfate and potassium hydroxide	Red leaflets or needles; m.p. 221° (decomposition)	(240, 336, 395)
$KCuC_2H_2N_3O_2 + 3H_2O$	Copper acetate and potassium hydroxide	Red-violet columns	(223, 240)
$[(C_2H_3N_3O_2)_2Cu]Na_2 + 2H_2O$	Copper acetate and sodium hydroxide	Bright pink precipitate	(313)
$[(C_2H_3N_3O_2)_2Cu]Ba + 2H_2O$	Copper hydroxide, potassium hydroxide, and barium hydroxide	Red crystals	(394)
$[(C_2H_3N_3O_2)_2Cu]Tl_2$	Copper acetate and thallous hydroxide	Red crystals	(393)
$[(C_2H_3N_3O_2)_2Cu][Ag(NH_3)_2]_2$	Copper hydroxide, silver oxide, and ammonia	Ruby-red prisms	(392)
$[(C_2H_3N_3O_2)_2Cu][Cu(NH_3)_4] + 3H_2O$	Copper hydroxide and ammonia	Reddish-violet prisms	(391)
$[(C_2H_3N_3O_2)_2Cu][Ag(en)_2]_2$	Copper hydroxide, silver oxide, and ethylenediamine	Ruby-red columns	(392)
$[(C_2H_3N_3O_2)_2Cu][Cu(en)_2] + H_2O$	Copper ethylenediamine hydroxide	Purple needles; m.p. 198°C.	(391)
$[(C_2H_3N_3O_2)_2Cu][Zn(en)_3] + 4H_2O$	Copper hydroxide and zinc ethylenediamine hydroxide	Red needles or plates	(395)
$[(C_2H_3N_3O_2)_2Cu][Cd(en)_3] + 4H_2O$	Copper hydroxide and cadmium ethylenediamine hydroxide	Wine-red crystals	(395)
$[(C_2H_3N_3O_2)_2Cu][Ni(en)_3] + 4H_2O$	Copper hydroxide and nickel ethylenediamine hydroxide	Red needles	(395)
$[(C_2H_3N_3O_2)_2Cu][Co(en)_3] + 4H_2O$	Copper hydroxide and cobaltiethylenediamine hydroxide	Reddish-brown crystals	(395)
$[(C_2H_3N_3O_2)Cu][(CH_3)_4N]_2 + 4H_2O$	Copper hydroxide and tetramethylammonium hydroxide	Red prisms	(395)
$[(C_2H_3N_3O_2)_2Cu][CH_6N_3]_4 + 2H_2O$	Copper hydroxide and guanidine	Red needles	(395)
$[(C_2H_3N_3O_2)_3Cu][(C_2H_5)_4P]_2 + 4H_2O$	Copper hydroxide and tetraethylphosphonium hydroxide	Red needles	(395)
$[(C_2H_3N_3O_2)Cu][(C_2H_5)_3S]_2 + 4H_2O$	Copper hydroxide and triethylsulfonium hydroxide	Reddish-blue crystals	(395)
$[(C_2H_3N_3O_2)_2Cu][(C_6H_5)_4I_2] + 4H_2O$	Copper sulfate and diphenyliodonium hydroxide	Brilliant red crystals	(393)

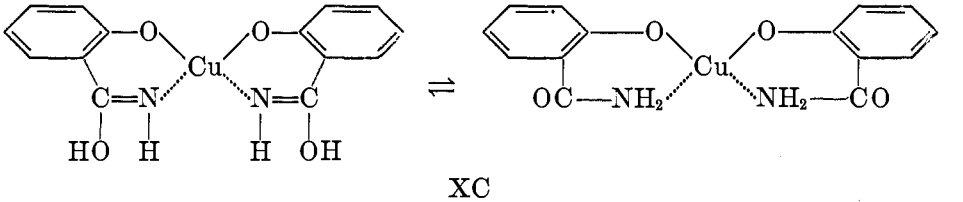
Biuret–metal complexes may be isolated in the form of well-defined crystalline solids when their solutions are carefully evaporated under reduced pressure. Being fairly labile, they are recrystallized only with difficulty. They absorb carbon dioxide from the atmosphere and are discolored more or less rapidly by the metal hydroxide or oxide thus liberated (333, 395); they must therefore be stored in an inert atmosphere. The complex salts are, of course, instantly decomposed by the addition of mineral acids (e.g., two equivalents of 1 *N* hydrochloric acid) (336); the copper, now present in the cationic form in the de-

TABLE 11

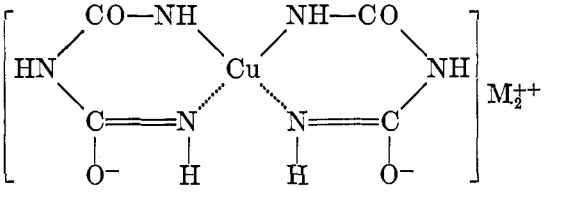
Biuret–nickel complexes

Compound	Reagent	Appearance	References
$[(C_2H_3N_3O_2)_2Ni]K_2$	Potassium hydroxide and nickel salt	Yellow solid	(333, 395)
$[(C_2H_3N_3O_2)_2Ni]Ba + 4H_2O$	Barium hydroxide and nickel hydroxide	Yellow crystals	(394)
$[(C_2H_3N_3O_2)_2Ni]Tl_2$	Thallous hydroxide and nickel acetate	Yellow crystals	(393)
$[(C_2H_3N_3O_2)_2Ni][Ag(NH_3)_2]_2 + 6H_2O$	Silver oxide, ammonia, and nickel sulfate	Yellow crystals	(395)
$[(C_2H_3N_3O_2)_2Ni][Cd(en)_3] + 4H_2O$	Cadmium ethylenediamine hydroxide and nickel hydroxide	Yellow needles	(395)
$[(C_2H_3N_3O_2)_2Ni][Ni(en)_3] + 4H_2O$	Ethylenediamine and nickel hydroxide	Yellow crystals	(395)
$[(C_2H_3N_3O_2)_2Ni][(CH_3)_4N]_2 + 4H_2O$	Tetramethylammonium hydroxide and nickel hydroxide	Yellow needles	(395)
$[(C_2H_3N_3O_2)_2Ni][CH_6N_3]_2 + 2H_2O$	Guanidine and nickel hydroxide	Yellow needles	(395)
$[(C_2H_3N_3O_2)_2Ni][(C_6H_5)_4I_2] + 4H_2O$	Diphenyliodonium hydroxide and nickel salt		(393)

colorized solution, may then be determined gravimetrically in the usual way (208, 336).

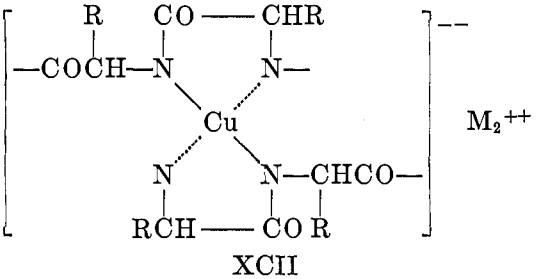


XC



XCI

(M = monovalent metal)



XCII

As a result of observations on coördination compounds of the type of XC derived from salicylic acid amide and related compounds, and from dipeptides,

Pfeiffer (286, 287) formulated the copper–biuret complexes as XCI. This structure accounts satisfactorily for all properties of these compounds and may be extended to the analogous products from polypeptides and proteins, which are represented as XCII.

The above hypotheses account satisfactorily for the general experience that 1-substituted biuret derivatives do not give the biuret reaction. Irrespective of the nature of their substituent (e.g., alkyl or aryl (335); acyl (271, 336); benzal (336); or nitro (90) radicals), the "blocking" of one of the positions potentially involved in complex formation prevents the synthesis of the colored products[5]). The argument applies, of course, even more forcibly to more highly substituted products, including 1,1-disubstituted (381) and 1,5-disubstituted (333, 336) derivatives, none of which give the biuret reaction. On the other hand, complexing in accordance with the above mechanism is not impeded by the replacement of the central hydrogen atom in 3-substituted biurets (40, 333, 335), which are capable of producing the violet biuret color in the usual way (285). Similarly, in agreement with expectation, the presence of a thiocarbonyl group (e.g., in thiobiuret) (333) does not prevent the biuret reaction from taking place. However, the easy formation of six-membered internal complexes cannot be envisaged in the case of triuret, which is known to be incapable of forming the typical copper complexes (335).

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